

Even misconduct trials should be fair

The US National Institutes of Health have been compelled to put out their rules for investigating misconduct in research for public comment. Not before time.

THE troubling issue of misconduct in science is in the news again. A draft report by the US National Institutes of Health (NIH) in the five-year-old dispute about data in a paper by Tufts University researcher Thereza Imanishi-Kari has been leaked, leading to widespread reports that the NIH Office of Scientific Integrity (OSI) has found Imanishi-Kari guilty of fabricating data in a paper of which Dr David Baltimore and others were also authors. According to OSI procedures, the principals now have several weeks in which to review and, possibly, rebut the draft report, whose pages are stamped "confidential" and "subject to verification and correction", before NIH issues a final opinion in the case. The matter of Imanishi-Kari's guilt or innocence aside, last week's events are sure to raise anew questions about the way OSI conducts its business, particularly with respect to fairness to the accused.

Sometime within the next few weeks, NIH will lay out for all to see the rules they follow when investigating allegations of scientific misconduct. What we shall see is that the two-year-old OSI denies the accused persons the right both to see at first hand the evidence against them and to confront their accusers face to face. In the Imanishi-Kari case and others, for example, OSI has refused requests by the accused to cross-examine witnesses. The issue is both a constitutional question of due process and one of simple fairness: OSI's proceedings can result in a denial of research funds to the accused, even dismissal.

Truth

NIH's integrity office, solicitous of the anonymity of accusers (or perhaps whistleblowers), argues that because a scientist has no constitutional right to federal funds, there is no constitutional right to due process in an investigation of the honesty of research conducted with those funds. Indeed, OSI officials like to think of themselves as scientists in pursuit of scientific truth, not as investigators of alleged fraud. To that end, they operate as if their office were a debating society. "In science, the burden of proof is on the person challenged", OSI has said. So those accused need not see the primary evidence against them, but merely need to prove scientific truth, responding not to data but to OSI's interpretation of them. OSI interviews witnesses, examines notebooks and scientific papers (and in the Imanishi-Kari case, conducted forensic analyses of data tapes) and then "frames the issues" to which a defendant must respond. Interestingly, in this case, two members of the scientific panel OSI appointed as advisers

have formally dissented from the OSI's interpretation of the data, which suggests that OSI's framing is not always unambiguously correct.

This approach is ripe for vigorous and open debate, which it will soon get, thanks to a federal court in Wisconsin where a defendant, neurologist James Abbs, sued NIH (see *Nature* 350, 100; 14 March 1991). Abbs had been accused of forging graphs in a 1987 paper, essentially by "plagiarizing" his own previous work. He took NIH to court on the grounds that he had been denied due process. He lost on substance, but won on a procedural technicality: OSI failed to publish its procedures for investigation before finally adopting them, thereby violating a law that gives the public the right of prior comment. NIH have now been forced back to the drawing-board: OSI is now putting its procedures out for public comment.

When this happens, OSI is likely to be attacked not only for due process but also for what lawyers consider its fuzzy standards of evidence used in deciding whether to begin or conclude an investigation. How much evidence is needed, and of what sort, to justify subjecting someone to months of quasi-legal probing? At what point does the absence of conclusive evidence bring matters to a close? What are OSI's standards for distinguishing fraud from lesser faults? OSI is under considerable political pressure to find people guilty, lest it be accused of whitewash. Thus, in its zeal to outdo Sherlock Holmes, it leaves no datum unturned, sometimes giving the impression that it is pursuing impolite or careless behaviour as zealously as data fabrication.

The continuing case of Robert C. Gallo is relevant. NIH have launched an OSI probe of Gallo's research on the AIDS virus, based on a newspaper account that reviewed an old dispute with French scientists. NIH officials, French officials and New York attorneys who represented the French in a settlement signed by the US and French heads of state all declared the new account contained nothing they did not already know. Lawyers rightly ask then about standards of evidence.

In matters as potentially ruinous to a researcher as a misconduct investigation, justice delayed is more than ordinarily justice denied. In the United States, NIH require universities at which accusations of misconduct have arisen to complete an "inquiry" within 60 days — 60 days to decide whether there is cause to proceed with a formal "investigation". Once an inquiry becomes an investigation, certain procedural safeguards come into force; records of interviews must be kept,



and the accused has a right to an attorney (whose fees the accused must pay), for instance. OSI itself, by comparison, moves at a snail's pace. It may spend months on what it fancifully calls a fact-finding "inquiry" before deciding whether to move to the more serious stage of an "investigation".

When, after "inquiries" that can include the interrogations of numbers of laboratory workers, OSI announces an "investigation", the accused is inevitably burdened with a presumption of guilt. Even OSI's advisers have been perplexed by its insistence that a full court analysis of every notebook in a person's possession, together with extensive interviewing of laboratory workers, can constitute a mere inquiry. Again, the Gallo case is pertinent. Gallo himself, as well as certain of his laboratory staff, submitted to more than 100 hours of "interviews" with OSI during what OSI said was nothing more than a preliminary fact-finding exercise. One cannot help but think of Alice in Wonderland's friend Humpty Dumpty who said, "When I use a word it means just what I choose it to mean — neither more nor less".

To be fair, OSI's procedures have developed as they have so as to preserve some sort of academic atmosphere while keeping lawyers at arm's length. Well-intentioned, to be sure. But OSI's powers are so close to those of a prosecutor and jury combined that it may be time to face the implications of that reality. Lawyers who have represented accused scientists have said that their clients, even if found guilty, would have been better off in court than in the hands of NIH.

The matter of the leaked report in the Imanishi-Kari case is an example. OSI argues that it adheres to due process and fairness in principle because the accused has a chance to rebut the OSI's conclusions before they are made final. And, to be sure, OSI asks those who receive copies of its draft conclusions to maintain their confidentiality. That is part of what OSI claims to be due process. But it is plain that OSI cannot enforce its confidentiality request. In the same week that the Imanishi-Kari draft was leaked, someone else leaked a report on a case involving a Georgetown University scientist to the *Washington Post*. It is understandable that lawyers protest that their clients are found guilty in the press before the proceedings are over. Were this taking place in the judicial system, violation of a confidentiality provision would lead to contempt of court and stiff fines.

The issue of fair proceedings has been festering long enough. It is good that the process is now to be scrutinized. Perhaps the whole process of investigating allegations of misconduct would be fairer in the long run were it to be fully open to public scrutiny as well — certainly once past the 60-day inquiry stage. The argument that private proceedings protect the reputation of the accused but innocent scientist does not hold water. In truth, once the fact that OSI is investigating someone becomes known, the accused's reputation is tarnished. In a closed system, it is therefore nearly impossible to undo the damage the way an acquittal does in an open court. Similarly, it is difficult for the scientific community to evaluate the seriousness of an OSI guilty verdict because the important details on which guilt or innocence may hinge are never laid out.

Investigations of scientific misconduct should be subject to

the "sunshine" laws that apply to many areas of government business. NIH should develop a system whereby the prosecutor — OSI — and the defendant could present their respective cases to an appropriately constituted panel of peers in an open hearing.

Because OSI is a quasi-legal office, it should in fairness adopt the safeguards of the legal system. □

Minister's reply

An innovation in relations between the British government and its critics is not an immediate success.

SQUABBLING about the adequacy of research funds is a long-standing British tradition which has reached a pinnacle of sophistication this week, involving the distinguished Select Committee on Science and Technology of the House of Lords and the rumbustious Secretary of State for Education and Science, Mr Kenneth Clarke. At the weekend, the select committee put out its most recent report on the state of British science, in particular pleading that there should be an immediate increase of £12 million in the funds at the disposal of the Science and Engineering Research Council (SERC), the largest of the five research councils and that most badly affected by this year's shortfall. Then, on Monday morning, before people would have had a chance to learn what the House of Lords was saying, the *Daily Telegraph* published a characteristically robust statement by Mr Clarke insisting that the British government is doing its duty by research. The newspaper, rather than the minister, linked together the report and his statement, which is not a direct reply.

There is, of course, no reason why ministers should not defend their departments against criticism in whatever ways seem appropriate. It is also much to be welcomed that they should do so by writing in the newspapers. (Mr Michael Foot MP, an employment minister in the 1970s, did just this to considerable effect.) But it will be a daunting refinement of the political process if ministers take to anticipating hostile comments on their conduct of their briefs and to disarming them with simultaneous publications in the public prints.

In reality, the select committee's criticism has not been disarmed. The essence of its case is that the increase of the science budget in the year beginning next month, nominally 2.7 per cent, is not enough to keep pace with inflation (most recently 8 per cent), and can be made to seem the 6 per cent the government claims only by an accounting device (postponing part of the cost of this year's graduate students until next year). Clarke's line is that "no country can afford to spend an unlimited amount on science", that priorities are necessary and that, for his part, he will act on the basis of the advice by the Advisory Board for the Research Councils. That would be a more compelling tale if he would publish the advice. Meanwhile, everybody concerned should recognize that amounts of money matter less than the government's stop-go spending in the field. Shutting enterprises for lack of funds in a mere six months is not only expensive, but bad for the spirit. And that has been going on for ten years now. □

Helping Europe compete in human genome research

- More coordination needed
- A role for the ESF?

Munich & London

WITH the United States stepping up its financial commitment towards sequencing the human genome, European genome researchers are realizing that something must be done to ensure that Europe does not fall behind.

Just what should be done to improve the European effort was discussed in two reports published last week. Two studies commissioned by the German Research Minister Heinz Riesenhuber, from the Strasbourg-based European Science Foundation (ESF) and the London-based Academia Europaea suggest that many of the weaknesses of European genome research could be mitigated by better coordination of existing national programmes — coupled with more money.

Europe currently has six large human genome initiatives, run by the European Communities (EC), Denmark, Germany, France, Italy and the United Kingdom. The ESF and Academia reports agree that US spending is roughly three times that in Europe (although some European researchers say that the US budget includes old initiatives that have been pulled into the human genome project, so it is not all 'new money').

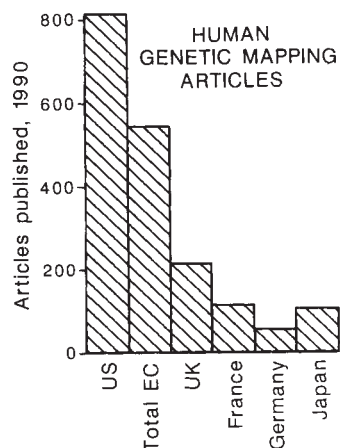
But Europe does not necessarily need to treble its genome budget to keep pace with the US effort. The ESF, an umbrella organization for European research councils and academies, offers one prescription that would cost little but could mean a lot: more cooperation, collaboration and coordination.

That however leaves the question of who should do the coordinating, and here the ESF study ventures into controversy. The Human Genome Organization (HUGO) was set up in 1988 with the express aim of improving the coordination of the international efforts to sequence the human genome. But the ESF report says that HUGO "has still to publicly articulate its role" and charges that HUGO has too broad a mission to deal with many of the specifically European problems of coordination that will arise. Instead, the ESF might provide the best forum for discussing the coordination of European genome research, the report suggests.

Sir Walter Bodmer, president of HUGO, has reservations about the proposed role for the ESF. Because HUGO is an organization of scientists involved directly in genome research, it is best placed to provide 'bottom up' coordination of the European effort, he says. He also rebuts ESF's lack-of-articulation charge, contending that HUGO's aims are well known in the genome research community. Bodmer admits that ESF could

"play a helpful role on the funding side" by arguing the case for increased support for genome research with national governments and the EC, but he warns that the proposed role for the ESF should not conflict with HUGO's activities.

Besides chiding HUGO, the ESF report has some specific criticisms of the current EC effort. EC support for genome research is divided between a number of different programmes, with work on other species, including the mouse, *Drosophila* and yeast, funded separately from the two-year, 15 million ECU (about £10.5 million) human genome analysis programme. The ESF working party says that EC genome research



Despite worries about the funding and coordination of the European contribution to the human genome project, European genome researchers at present seem to be performing well. An analysis of the MEDLINE database included in the ESF report shows that US researchers accounted for the largest single share (46.8 per cent) of scientific papers on human genetic mapping published during 1990. But researchers from the European Communities came a close second, publishing 31 per cent of the total, dwarfing the output of Japanese researchers.

should be "integrated under a single umbrella".

But Bronwen Loder, who divides her time between running HUGO's London office and the EC human genome analysis programme, does not agree that there is a strong case for placing all EC genome research into one programme. The separate strands of EC genome research are well coordinated through a European Commission committee she says.

In contrast to the ESF report, which concentrates on research management, the Academia Europaea study focuses on the scientific aspects of studying the human genome.

The Academia, a fellowship of eminent European scientists, urges European groups to focus on four areas: genetic and physical mapping of human and other genomes; the identification of new methods to identify potentially important regions of the genome that may play a role in identifying the genetic basis of disease; the sequencing of single genes and the development of better sequencing technologies; and the setting up of databases to cope with the 'avalanche' of sequence information.

In commissioning the reports, Germany seems to be attempting to take the lead in organizing European genome research.

This development is ironic in two ways. First, (West) Germany was the main objector to the original EC human genome research programme, which carried the unfortunate title of "Predictive Medicine". That programme was withdrawn in 1988 after a storm of protest from the German government and from left-of-centre parties in the European Parliament. Both groups objected to what they saw as 'eugenic' tendencies in the original programme and a lack of sensitivity to ethical considerations (see *Nature* 336, 416; 1988). Riesenhuber hopes that the left wing will be mollified by the Bundestag's (German parliament) promise to address such uses of genomic data as employee screening and prenatal diagnostics in the current four-year legislative session.

The second irony is that Germany, despite having a national programme for genome research has devoted a relatively paltry sum to it. With a total 1991 commitment of DM27 million (about \$16 million), the amounts invested in Germany are "not comparable to what's going on in France or Britain", says John Collins, head of cell biology and genetics at the Institute for Biotechnological Research in Braunschweig.

One problem for Germany is the lack of qualified research personnel. Pohl puts it diplomatically when he says, "The number of first-class people doing this kind of work in Germany is not very large." Pohl was one of a group of researchers who in 1988 proposed a five-year, DM100 million programme for genome analysis in Germany, which was cut by the ministry to DM25 million and incorporated into an existing "Priority Programme" of the granting agency DFG.

Riesenhuber told *Nature* that the suggested programme was so badly slashed, in part because it seemed to come from a small group of researchers with little support from the general scientific community. But he asserts that there are plenty of good groups working on "problem-oriented" genome research, that is, sequencing single genes or gene complexes. And he promises that if having enough qualified personnel should turn out to be a problem, he would initiate a programme for young researchers to improve the situation.

Steven Dickman
& Peter Aldhous

NIH: Imanishi-Kari guilty

Washington

FIVE years after charges were first made, one of the longest, most painful misconduct cases in US science history may have reached its end. In a 280-page draft report, the US National Institutes of Health (NIH) have found that Tufts University biologist Thereza Imanishi-Kari fabricated key data in a 1986 *Cell* paper of which Nobel laureate David Baltimore, now the president of Rockefeller University, was also an author.

The report, which was leaked to journalists last week, reverses the conclusions of a previous NIH investigation and earlier inquiries at Tufts University and the Massachusetts Institute of Technology, where Imanishi-Kari had done the original work. It finds that data from two critical experiments described in the paper were "fabricated" and that Imanishi-Kari "repeatedly presented false and misleading information to the NIH" and other investigative panels to hide that fact.

Baltimore last week retracted the *Cell* paper and issued a statement saying that the NIH report "raises very serious question about the veracity of the serological data in the paper". And he added "It is up to Dr Imanishi-Kari to resolve them".

The principal finding of the paper — that transplanted genes influence a mouse's own genes — was based on data supplied by Imanishi-Kari that purportedly described experiments on transgenic mice. "It remains unclear if these experiments were actually done," the OSI report finds. "But if they were, the resulting data must have been deemed unreliable or otherwise unsatisfactory, since an attempt was made to substitute other data for them."

Imanishi-Kari, Baltimore and Margot O'Toole, the whistleblower in the case, have 30 days to respond to the draft report before it becomes final. Should the conclusions remain unchanged, the NIH Office of Scientific Integrity (OSI), which investigated the case, will recommend sanctions to NIH's parent agency, the Department of Health and Human Services (HHS). If OSI recommends that Imanishi-Kari be denied further federal research support — a typical response — HHS would begin a formal debarment proceeding.

In a worst-case scenario, such a procedure could take another two years while the case is heard again, something OSI deputy director Suzanne Hadley describes as "daunting to consider, but not impossible".

Although the report reviews the laboratory notebooks in exhaustive detail, it does not appear to make a completely airtight case for Imanishi-Kari's guilt. Secret Service analysis of certain handwritten notebook entries purportedly copied from gamma-counter readings of Imanishi-Kari's experiments showed a statistically improbable favouring of the numbers 1, 3, 7 or 8 in

the 'tens' digit.

The Secret Service determined that the odds of such 'spikiness' naturally occurring is less than 1 in 100,000.

But a dissenting opinion by Hugh McDevitt and Ursula Storb, two of OSI's five-member independent review committee, raises questions about the statistical analysis. Because such analysis is new to the investigation of scientific fraud and little work has been carried out on number distribution in scientific data, the two committee members felt that "we can only conclude that [the analysis] is interesting, but not compelling, and certainly not adequate grounds for an allegation or conclusion of fabrication".

They did, however, find the Secret Service forensic analysis of certain gamma-counter tapes in Imanishi-Kari's notebooks more convincing. Chromatographic comparison of the inks and print quality of the tapes against similar data from other researchers in the same MIT laboratory indicated that the tapes purportedly printed in 1985 experiments were actually made as much as three years earlier (see *Nature* 347, 317; 507, 1990). Even for these sceptical members of the panel, that analysis "makes it seem likely that the data on these pages are not the result of experiments performed at or near the time stated".

The dissenting opinion rejects five of the seven OSI conclusions of misconduct, arguing that the findings "are open to several alternative interpretations". But the opinion does not specify what those other interpretations might be, and both McDevitt and Storb declined last week to elaborate on their written statement.

Late last week, OSI sent a letter to parties in the investigation that revealed errors in the report. Tapes that were described in the report as green were actually yellow, OSI admitted. OSI said that the overall results nevertheless "stand as stated".

OSI intends to review also the Tufts and MIT investigations, to determine if they were handled correctly, Hadley says, although the office may not issue a formal report on that point. Congressman John Dingell, chairman of the House of Representatives Energy and Commerce oversight and investigations subcommittee, which has so far held four hearings on the case, intends to hold yet another hearing in late April or May. Peter Stockton, a subcommittee staff member, says the hearing will address the "who-knew-what-when" aspects of the earlier investigations. Baltimore and Imanishi-Kari will be called to testify, among other witnesses, Stockton says.

Brigitte Huber, a member of the Tufts committee that found no evidence of misconduct, says that lack of investigative resources had forced her committee to accept

Six years of intrigue

May 1985: Thereza Imanishi-Kari carries out transgenic mouse experiments at MIT.

April 1986: Original paper based on the mouse data is published in *Cell* by Imanishi-Kari, David Baltimore and others.

May 1986: Laboratory postdoctoral fellow Margot O'Toole discovers an Imanishi-Kari notebook containing 17 pages that suggested to her that some of the key experiments in the *Cell* paper had never been done; Tufts University, which is preparing to hire Imanishi-Kari, convenes an *ad hoc* committee headed by biologist Henry Wortis to investigate charges.

June 1986: MIT professor Herman Eisen meets O'Toole, Imanishi-Kari and Baltimore to review O'Toole's allegations. His memorandum finds possible minor errors, but no fraud.

October 1986: NIH researchers Walter Stewart and Ned Feder examine the 17 notebook pages and inform NIH officials that they suspect misconduct.

May 1987: NIH Office of Extramural Research begins first inquiry; Tufts committee submits report concluding that there was no deliberate falsification or misrepresentation in the *Cell* paper.

May 1988: Congressional Investigations Subcommittee of Representative John Dingell holds its first hearings, focusing on the response of Tufts and MIT to the O'Toole allegations; Baltimore issues a "Dear Colleague" letter attacking Dingell and asserting that congressional interference "is totally unnecessary".

July 1988: Dingell subpoenas Imanishi-Kari's laboratory records and turns the notebooks over to Secret Service for analysis.

November 1988: Baltimore and Imanishi-Kari publish a correction in *Cell* indicating that the original paper contained an "overstatement" of the specificity of BET-1, a key reagent.

January 1989: First NIH investigation ends, finding "significant errors of mis-statement and omission... [but] no evidence of fraud, conscious misrepresentation, or manipulations of data".

April 1989: Based on new evidence from the continuing Dingell investigation and subsequent O'Toole findings, NIH reopens the investigation within the newly created Office of Scientific Integrity.

May 1989: Dingell holds two hearings, at the first of which the Secret Service testifies that 20 per cent of a critical notebook is forensically questionable; on the direction of NIH, Baltimore and Imanishi-Kari publish a second correction in *Cell*, giving additional data on the specificity of BET-1.

May 1990: Dingell holds fourth hearing. Secret Service investigators present additional forensic data showing that Imanishi-Kari's notebook records and purported experiments were "not contemporaneous with respect to time". Findings cast doubt on the data in the second *Cell* correction.

March 1991: Draft report of second NIH investigation reverses previous report, finds "serious scientific misconduct", including data fabrication. **C.A.**

HUMAN EXPERIMENTATION

AIDS trials questioned

Imanishi-Kari's notebooks at face value. "There was no way for us to tell if the data were real — we couldn't analyse the counter tapes like the Secret Service," she says.

"Everything has changed so much in the last three years since the Tufts probe," Huber says. "Everybody is so much more sensitized. When these things come up now they're taken much more seriously." She points out that the committee on which she served was created informally in less than a week to examine O'Toole's accusations. NIH has since issued guidelines for the creation of formal university panels to investigate such cases of alleged scientific misconduct.

As evidence of misconduct emerged, Baltimore came under increasing fire for his steadfast refusal to accept the possibility that Imanishi-Kari had fabricated critical data in the *Cell* paper. Former NIH director James Wyngaarden admonished Baltimore and his co-authors in a 1989 letter: "[You] never met to consider seriously the allegations or to reexamine the data . . . Such an analysis . . . may well have made a full examination unnecessary." But Baltimore says that confusion in Imanishi-Kari's records was partly to blame. "The whole investigation has been very difficult because of the sloppy methods used," he says. "The evidence mounted, but I did not find it convincing. Until I was sure, I was not going to accuse her of something improper."

"Clearly the NIH has done a remarkable job" with the draft report, he says. "I find it much more convincing than anything previously said. The evidence that the gamma-counter tapes were produced before the mice were born is very disturbing."

OSI took a less charitable view of Baltimore. The draft report quotes him as accusing OSI of forcing Imanishi-Kari into misconduct by requiring that she publish additional data in the form of a correction to support the claims of the *Cell* paper. OSI has since concluded that the data in that correction were fabricated.

"... [I]n my mind you can make up anything that you want in your notebooks, but you can't call it fraud if it wasn't published", Baltimore told the OSI team of investigators. "Now, you managed to trick us into publishing — sort of tricked Thereza — into publishing a few numbers and now you're going to go back and see if you can produce those as fraud. But I think you should see that was a forced situation . . ." The draft OSI report describes that statement as "extraordinary".

Baltimore says that when he told the OSI team of investigators that researchers could ethically "make up anything" in their notebooks, he was not referring to data. He also distances himself from the rest of the statement. "It was clearly misconduct to discuss data that didn't exist", he explains. "It's not any less or more wrong because Thereza was forced to publish it."

Christopher Anderson

Washington

REPORTS that past US collaboration with French AIDS researcher Daniel Zagury (see *Nature* 350, 179; 21 March 1991) may not have complied with the requirements of US law on research with human subjects has led the US National Institutes of Health (NIH) to the unusual step of suspending all collaboration with Zagury and the institutions with which he is affiliated.

The allegations against Zagury have been made by journalist John Crewdson of the *Chicago Tribune*, and amount to the assertion that volunteers in work with putative AIDS vaccines had not been subjected to the full requirements of ethics committee review specified by US law. As long ago as 1987, Zagury reported the injection of a putative vaccine into himself.

US policy requires layers of approval for all collaborative research, whether it involves direct work with patients, the sharing of research materials or even testing of blood. NIH are in the midst of a broad examination of their policy on the ethical requirements of international research collaborations with human subjects.

A report on overall policy requirements, and a decision in the Zagury case, are expected soon. Meanwhile, NIH officials have banned all NIH scientists from collaboration with Zagury and with the institutions at which he is working on an AIDS vaccine. These include the Université Pierre et Marie Curie and its affiliated hospitals in Paris, the Cliniques Universitaires de Kinshasa in Zaire and the Institut National de Recherches Biomedicales in Zaire.

During the past several years, Zagury's collaborators in the United States have included Robert C. Gallo of the National Cancer Institute, other NCI researchers, Takas Papas, an NCI contractor, and Bernard Moss of the National Institute of Allergy and Infectious Diseases.

A preliminary NIH inquiry indicates, for instance, that before Moss sent Zagury recombinant vaccinia incorporating the *env* gene from Gallo's widely distributed IIIB strain of AIDS virus, appropriate ethical approvals were in hand from the allergy institute's committee, the French ethical committee and the US Food and Drug Administration, which authorized shipment of vaccinia in response to a request from the French National Health Laboratory.

However, informed consent documents may not have been approved by all the committees specified in the law.

Another allegation is that Gallo tested blood samples that Zagury drew from military personnel in Zaire without asking for the approval of the ethical committee at NIH. Gallo says, "This is true. I didn't know you had to have approval to study blood samples even if you were not involved in the clinical research." Zagury did have approval from

officials in Zaire. NIH ethics officials say they now realize that there are very few NIH researchers who know that, particularly those whose research does not bring them into contact with patients.

It is also alleged that NIH are in violation of their own rules because their researchers sent Zagury materials intended for animal and laboratory studies that were actually used in human vaccine trials. This Zagury vigorously denies. In a 6 March 'certificate' to the NIH, he wrote "I never administered to human individuals during clinical trials performed in Zaire or in France any reagents provided by the NCI".

Crewdson's evidence to the contrary appears in a *Chicago Tribune* article that, referring to Zagury's most recent study, quotes one participant as saying that the husband of Zagury's principal collaborator had said, "the stuff we were being injected with was from Gallo's laboratory".

Perhaps the most serious ethical question goes back to Zagury's experiments in 1986 and 1987 with an early AIDS vaccine preparation that he tried out on himself and on what he described (in *Nature*) as "a small group of Zairians, all of whom were HIV-seronegative volunteers and immunologically normal . . ." (*Nature* 326, 249; 1987).

What the paper fails to mention, it now emerges, is that the Zairian volunteers were all young children whose fathers had died of AIDS and whose mothers were infected. Zagury says he obtained informed consent from the mothers, who "begged" him to protect their children. NIH officials say it is doubtful the experiment would have won ethical approval, based on what they know now.

NIH's own report says that, in 1986, Moss sent Zagury "a large quantity" of recombinant vaccinia virus (V25) for use in animals. It is now reported that Zagury used this recombinant vaccinia virus to make his first human AIDS vaccine, a fact that Moss reported to the NIH's OPRR. The NIH preliminary report says only that "OPRR is aware of Dr Zagury's unauthorized use of the vaccinia virus in humans. Therefore, we will not discuss it further." OPRR officials decline to comment on their own behaviour.

But they have appointed a committee to look into the current case, naming Seymour Klebanoff of the University of Washington in Seattle as its chairman. Members of that committee are reported already to have submitted their report to OPRR. One of them noted privately that the real challenge is sorting out procedures in which no ethical standards were violated, even though appropriate committee review was not secured, from those in which the requirements were effectively ignored. Nevertheless, in the final analysis, NIH are going to have to put their house in order and make their rules unambiguously clear.

Barbara J. Culliton

Lords ask for crisis handout

London

THE government should release £12 million immediately to prevent the cuts in research grants and studentships in 1991–92 announced by the Science and Engineering Research Council (SERC) last month, says the House of Lords Science and Technology (S&T) Committee. SERC chairman Sir Mark Richmond has said that SERC will award only 50 per cent of the usual number of new research grants this year, and 85 per cent of the studentships (see *Nature* 349, 551; 14 February 1991).

The S&T committee has been examining the circumstances that led SERC to cut its programme savagely, following the announcement of the 1991–92 science budget. Its report, published last Sunday (24 March), puts the heaviest burden of blame for the current crisis in the research councils onto the government: "The 1991–92 science budget falls far short of what is required to provide the basis for the continuing development of the country's science base."

The report says that the Advisory Board for the Research Councils (ABRC) had as-

sumed that much of the £23.3 million of capital expenditure in 1990–91, on the research ship *James Clark Ross* and a new research councils headquarters building in Swindon, would automatically become available to be spent on research this year. Sir David Phillips, chairman of the ABRC, told the S&T committee earlier this month that a number of new research programmes had been launched on this assumption, but the government had then decided that this money should not be built into the research councils "baseline" funding. The consequences of this decision are serious for all the research councils, the report says — all five have been forced to trim their future spending plans.

Somewhat surprisingly, however, the S&T committee's report is lenient in its criticism of SERC under its former chairman, Sir William Mitchell. Mitchell committed SERC to spending on a number of large projects, beyond the planning figures given by the government, in the hope that some £40 million of new money would be made available to SERC in 1991–92. In the event, SERC re-

ceived only £12 million. In 1991–92, research grants and studentships, as the only item of SERC's expenditure which can be cut easily, have suffered heavily.

The £12 million emergency fund requested in the report would reinstate these grants and studentships, described by the committee as "the very seed corn of the United Kingdom science base". But the report warns that the fund will provide only a temporary solution to what is a serious underlying problem. The Universities Funding Council's (UFC) spending on research has been falling steadily since 1987–88, the report says. This has meant that the total funding for academic science has fallen in each of the last two years. The S&T committee says that the government should use the "period of grace" allowed by the £12 million emergency fund to rethink its policy on science, and suggests a number of changes.

The Cabinet committee on science and technology, which is chaired by the Prime Minister, should involve itself more heavily in deciding the level of funding for the science base, the report says. The committee is worried that purely financial considerations dominate over scientific strategy in the annual discussion between the Treasury and the Department of Education and Science. The advice given to the Education and Science Secretary by the ABRC on the size of the science budget should be published to allow informed debate on science funding, according to the S&T committee. (This practice, established in the early 1980s, was abolished last year.)

The S&T committee is also concerned that the planned transfer of some £100 million a year of the UFC's spending on research to the research councils, which begins in 1992–93, will undermine the universities' ability to support young researchers. This £100 million is supposed to pay for the indirect costs associated with research-council-funded projects in higher education institutions. But these costs have proved difficult to identify, the committee says. Unless a fully workable scheme can be put in place soon to ensure that the transferred money is actually used to support research in the universities, the transfer should not go ahead, the report says.

But the government has so far shown no willingness to provide the £12 million emergency fund, let alone launch a wholesale review of its science policy. Education and Science Secretary Kenneth Clarke last week expressed sympathy with young researchers who will be denied research grants in the coming year, but told the S&T committee that the government could not bail SERC out. Otherwise, the other research councils would also start overspending, he said.

The research councils' financial problems were also compounded last week by a change in British taxation. Chancellor of the Exchequer Norman Lamont has added 2.5 per cent to Value Added Tax, a surcharge on purchased goods, which will increase the cost of scientific equipment.

Peter Aldhous

Confusion surrounds the 'Gardner effect'

London

THE mystery surrounding the occurrence of clusters of childhood leukaemia cases around some British nuclear plants deepened last week. A team led by James Urquhart, of the Scottish Health Service in Edinburgh, found that the fivefold excess of leukaemia cases around the Dounreay nuclear fuel reprocessing plant in Caithness, Scotland, cannot be explained by the exposure of the leukaemic children's fathers to radiation at work.

Last year, a group headed by Martin Gardner, from the University of Southampton, found a strong association between the external doses of radiation received by men working at the Sellafield nuclear processing plant in west Cumbria before the conception of their children, and the likelihood of these children developing leukaemia (see *Nature* 343, 679; 1990). This was a controversial finding, as there is no known mechanism to explain the effect, and because no excess of leukaemia cases has been found in the children of Japanese atom bomb survivors, whose radiation exposure was higher than that of the Sellafield workers.

Urquhart's study, published in the *British Medical Journal*, looked at 14 children who developed leukaemia or non-Hodgkin's lymphoma and 55 healthy controls. With such a small sample, the team say that they cannot confirm or deny the existence of the "Gardner effect". But the new Dounreay results show that Gardner's hypothesis does not explain the prevalence

of childhood leukaemia in Caithness.

Urquhart's team did find that children who had played on the beaches around Dounreay were more likely to develop leukaemia. But the researchers believe that this result may be an artefact of repeated statistical significance testing, or may have been affected by biased questionnaire responses from the parents of leukaemic children.

A second study published in the *British Medical Journal* last week adds to the confusion. Patricia McKinney, from the University of Leeds, and colleagues have looked at the parents of children with leukaemia in west Cumbria, north Humberside and Gateshead, all in the north of England, to identify any links between parental occupation and the occurrence of leukaemia in children.

McKinney's team found that a number of parental factors correlated with the occurrence of leukaemia, including paternal exposure to radiation. The factor most strongly associated was the parents' exposure to wood dust at work — a factor never before implicated in the disease. But McKinney's team did not quantify the parents' exposure to each of the factors they considered. In an accompanying leading article, Peter Smith, from the London School of Hygiene and Tropical Medicine, says that the study should be seen as generating some interesting hypotheses to be tested further, rather than providing strong evidence of new causative factors.

Peter Aldhous

NUCLEAR ENERGY

German reactor closed

Munich

THE second of three pillars supporting the German national nuclear power programme toppled last week as the German government decided to abandon its prototype fast-breeder reactor at Kalkar.

Research and Technology Minister Heinz Riesenhuber made the surprise announcement of dropping Kalkar last week after meeting with representatives of the builder of the plant, Siemens AG, and the three utilities that were going to operate it. Now those utilities are expected to try to turn the 300-MW plant, located 70 km west of Düsseldorf near the Dutch border, into an oil-burning facility in order to recoup some of their losses.

The Research and Technology Ministry, which paid more than 40 per cent of the DM7,000 million (about \$4,300 million) it cost to build the plant, will not be so lucky. All its investment must be written off as loss. And Belgium and the Netherlands, which each paid DM470 million before pulling out of Kalkar in 1983, are in the same boat. Riesenhuber said he no longer expected them to ask for their money back.

Before unification, West Germany derived roughly 38 per cent of its electricity from nuclear power, and for years it had planned to become independent of other countries by building its own fast-breeder and reprocessing facilities. But in 1989, Germany decided to abandon its controversial nuclear fuel reprocessing facility at Wackersdorf, which had already cost the government and utilities over DM2,000 million. It shifted its reprocessing needs to the French facility at La Hague. The latest decision leaves Germany with power reactors and nothing else.

The Kalkar plant was effectively completed by 1986. Riesenhuber blamed the Social Democratic (SPD) government of the

Land of North Rhine-Westphalia (NRW), in which the plant is located, for refusing to license it despite a clean safety record in the pre-operative phase. SPD has an absolute majority in NRW and has opposed nuclear power since the Chernobyl accident in 1986. The NRW government issued 17 partial licences before refusing in 1987 to issue the final licence for nuclear operation. The licensing procedure has been stalled ever since, during which time the reactor has cost Bonn DM10 million a month to maintain.

In capitulating to the SPD instead of taking the licensing decision to court, the centre-right coalition in Bonn recognized the power of SPD, the second largest party following Helmut Kohl's Christian Democrats, to influence Germany's nuclear course. This could become significant in deciding whether to build new nuclear generating capacity for the energy-poor eastern regions of Germany. The loss of Kalkar moved the chairman of VEBA AG, a parent company of one of Germany's largest utilities, to announce that he would not try to obtain permission to build or operate a nuclear plant in eastern Germany without the explicit approval of SPD.

Siemens and the Swedish-Swiss conglomerate Asca Brown Boveri are seen to have the inside track on construction of new plants in eastern Germany, which could rejuvenate an otherwise nearly stagnant European market for new nuclear plants.

The government decided to abandon Kalkar during coalition negotiations following elections on 2 December 1990. Riesenhuber had promised a final decision by the end of 1991, when public funds were due to run out. It was a surprise that the decision came so early in the year; the move was apparently justified by the shortage of funds brought about by reunification.

Steven Dickman

ANTARCTICA

Reprieve for French polar expeditions?

Paris

THE French minister for overseas departments and territories (DOM-TOM), Louis le Penec, has promised that the French polar expeditions (EPF) will not have to close their research base in Antarctica next year as feared.

A cash crisis in the ministry, worsened by recent budget cuts, had threatened that EPF's annual grant of FF26 million would have to be cut to FF20 million (see *Nature* 349, 553; 14 February 1991, and see page 299). This, said EPF's secretary, Bernard Morlet, would not be enough to enable both the 1991-92 summer and winter programmes to go ahead. And it could mean closing the Dumont d'Urville base for a year.

But last week, saying that he was "astounded by the various reports concerning

the future of EPF", le Penec announced that he would "reequilibrer" funds for EPF "in order that the 1991-92 winter programme may be launched without delay".

At EPF, however, it appears that the DOM-TOM promise has still not been translated into cash. "We are still a long way from our goal", says Morlet. Meanwhile, the ministry for research and technology (MRT) — which co-funds Antarctic research — is still at loggerheads with le Penec over DOM-TOM's contribution to the French polar research budget. There are fears that, if the Dumont d'Urville base is not closed, sacrifices will have to be made elsewhere. The clash, at a time when France is trying to expand polar research, is adding fuel to efforts to give MRT entire responsibility for financing Antarctic science.

Peter Coles

NEWS IN BRIEF

US opens pockets to protect species

Washington

FOR nearly a decade, the mantra of the ecology community has been the same: biological diversity worldwide is threatened and requires protecting in developing countries. Finally, it appears, the message has got through, at least in the United States. US public and private funding for projects to preserve biological diversity has increased by two-thirds in the past two years, according to a new report* by the World Resources Institute (WRI). Federal funding for biodiversity research climbed to \$23.1 million in 1989, an increase of 16 per cent over 1987. And for the first time, WRI found that contributions from private foundations have nearly matched the federal totals, with a total of \$21.4 million in 1989 — a 750 per cent jump over two years.

Thirty-eight per cent of the 1989 funding supported research programmes on maintaining a healthy mix of plants, animals and natural habitats in developing countries. Another 25 per cent went to species and site-management projects, whereas 'debt-for-nature' swaps accounted for about 5 per cent of the funding, WRI found.

C.A.

*Investing In Biological Diversity — US Research and Conservation Efforts in Developing Countries, World Resources Institute, March 1991.

New president

Boston

AFTER a prolonged selection process, Harvard University chose a new president last week: Neil Leon Rudenstine, a literary scholar and former administrator at Princeton University. Geneticist Philip Leder is known to have been a top contender for the job, and it is widely speculated that a deadlock in the search committee between Leder, Rudenstine and a handful of other finalists forced the panel to postpone their decision for weeks. Leder would have been the first scientist to head Harvard in nearly 40 years.

Rudenstine, meanwhile, brings to the job a background in the humanities and a proven record as an administrator. His style is said to be "conciliatory", and he is seen as a popular choice among students. He will succeed Derek Bok who steps down this June after two decades as Harvard's president.

S.S.

CORRECTION

The article on the European Research Conference "Gordon conference clones" (*Nature* 350, 179; 21 March 1991) may have created the impression that the conferences have not yet begun. In fact, the so-called "pilot phase" of the conferences, run by the European Science Foundation (ESF) with some support from the European Communities (EC), began in 1990 with six conferences and continues in 1991 with 23 more.

Going for 'green technology'

Tokyo

HAVING decided that environment-friendly technology will drive the future economic growth of the world, Japanese industry and government are pouring hundreds of millions of dollars into an effort to establish themselves as first in the field.

As with Japan's past successes in a number of industries, such as chip-making and home electronics, the powerful Ministry of International Trade and Industry (MITI) is the prime backer of the research and development of 'green technology', although funds are also coming from a wide spectrum of Japanese industry and academic institutions.

The past couple of years have seen remarkable growth in government support for the development of such products as biodegradable plastics, substitutes for ozone-destroying chlorofluorocarbons (CFCs), technology to absorb and utilize carbon dioxide, and biotechnology to mop up oil spills.

MITI's push actually began quietly in 1987 — before the global environment became a hot political issue — with a move to establish two semi-private marine biotechnology laboratories to develop useful products from the sea (see *Nature* 333, 4; 1988). The Shimizu and Kamaishi marine laboratories on the Pacific coast of Japan opened last April.

The two laboratories were built at a cost of ¥6,000 million (\$44 million) with investments and loans from 36 companies, city banks, local governments, the Japan Development Bank, the Hokkaido Tohoku Development Finance Public Corporation and MITI's New Energy and Industrial Technology Development Organization (NEDO).

NEDO will provide an additional ¥15,000 million in operating costs over nine years, and another ¥7,850 million will be provided over the same period by 24 participating companies, including shipbuilding, chemical, steel, oil-refining, construction, mining, brewing and paint companies.

The laboratories' original purpose was to isolate new pharmaceuticals and natural anti-fouling agents (for incorporation into ship paint) from marine organisms. But with the rising concern over the global environment, the laboratories are now switching attention to isolation of microorganisms that absorb carbon dioxide or eat up oil spills (see box, next page).

Coming close on the heels of the two marine laboratories will be MITI's Research Institute of Innovative Technology for the Earth (RITE). The institute, which will open next year in Kansai Science City between Osaka, Kyoto and Nara, is likely to be the largest project ever funded by MITI.

Sixty major companies are investing ¥5,000 million (\$37 million) in RITE, including Kansai Electric Power Company, Tokyo Electric Power Company, Tokyo Gas, NEC, Hitachi, Nissan, NKK, Hankyu Railway Corporation, Kobe Steel, Kawasaki Heavy Industries, cement and construction companies, chemical concerns, trading and life insurance companies, trust banks, and even Wacoal Corporation, Japan's leading manu-

facturer of women's underwear.

Local governments in the Kansai area will contribute another ¥3,000 million and provide free land for the institute. And NEDO is pouring in about ¥5,000 million a year to support RITE research, which is being carried out at private companies and MITI's national laboratories until the institute opens.

Like the Shimizu and Kamaishi laboratories, RITE will put a lot of effort into developing biological techniques for absorbing carbon dioxide. But the institute will also investigate chemical processes for carbon-dioxide absorption and will develop substitute

MITI's GROWING BUDGET FOR THE GLOBAL ENVIRONMENT

	million yen	
	1990	1991
RITE		
CO ₂ absorption:		
biological	1,134	1,315
chemical	581	900
Substitute CFCs	361	1,087
Biodegradable plastics	180	200
Bioreactor	72	72
Biological hydrogen production	0	42
Metallic scrap recycling	0	75
Joint research with private sector	2,000*	1,000*
Grants for basic research, symposiums and data bases	122	209
Surveys of global environment problems	80	100
TOTAL for RITE	~4,000	~5,000
Shimizu and Kamaishi marine biotech labs	1,186	1,390
Grants for MITI national labs	165	203

*Matched by an equal investment from private sector. Not all of the money assigned by MITI necessarily goes to RITE.

CFCs and biodegradable plastics.

Jiro Kondo, president of the Science Council of Japan and director of the RITE laboratory, says he is pleasantly surprised by the "strong support" industry is giving to what will probably be fairly long-term basic research. And he says that the presidents of Kansai and Tokyo electric power companies, two of the largest private electric power companies in the world, and the head

US—Japan collaboration to develop oil-eating bacteria

Tokyo

THE Japanese are not going it alone in their quest for environment-friendly technology. US and Japanese researchers recently announced an unprecedented joint effort to genetically engineer microorganisms that can mop up oil spills and degrade other man-made chemical pollutants.

The five-year \$15-million project, which will be funded equally by the US National Science Foundation (NSF) and the Research and Development Cooperation of Japan (JRDC), is the first major project to come under the umbrella of the 1988 US—Japan Science and Technology Agreement and is indicative of a new cooperative spirit in basic research between the two rival economic giants.

The microbe project will be led by James Tiedje, director of Michigan State University (MSU) Center for Microbial Ecology, and Kieji Yano, professor of biology at Nagaoka University, a leading

authority on the molecular biology of genes involved in biodegradation. The research will be carried out by 20 researchers, half of whom (five Americans and five Japanese) will be based at MSU. The other half will work at Nagaoka University of Technology and the Institute of Physical and Chemical Research (RIKEN) in Japan.

The project is the first US—Japanese collaborative effort under a new programme for joint international research introduced by JRDC, an affiliate of Japan's Science and Technology Agency.

Japan's perceived lack of contribution to basic research has been a serious source of friction with the United States that flared up during renegotiation of the US—Japan science and technology agreement in 1988. But the United States has recently been showing a tendency to collaborate in Japan's efforts to promote international basic research. For example, Japan's Human Frontier

Science Programme will receive some financial support (albeit small) from the National Institutes of Health (NIH) (see *Nature* 350, 97; 1991). And the new microbe project is also indicative of a new willingness on the part of the United States to contribute to international Japanese projects.

Tiedje says the research is based on the fact that microbes ultimately consume or degrade all natural organic products, from leaves that fall in a stream to garbage in landfills. He and his Japanese collaborators believe that genetic engineering and an understanding of microbial evolution can be used to produce microbes that can degrade oil spills and other man-made pollutants such as PCBs.

The project provides for "equitable sharing of resources and expertise as well as equitable sharing of patents and other results of economic importance", Tiedje says.

D.S.

of the powerful Kansai Economic Federation are being "very cooperative".

Japan's electric power companies are particularly keen to develop carbon-dioxide-absorbing technology because they account for about 30 per cent of Japan's total carbon-dioxide emissions. Heavy industry and chemical companies are also hoping to build carbon-dioxide-absorbing plants for their own use and for sale, while trading companies hope to export the plants overseas.

The push to develop environment-friendly technology is not confined to RITE and the marine laboratories. A small but significant effort is under way at the Department of Biotechnology of Tokyo University of Agriculture and Technology led by Tadashi Matsunaga and backed by industry. Researchers there hope to develop a 'biosolar reactor' that will use genetically engineered photosynthetic microorganisms to absorb carbon dioxide and produce useful extracellular products — a project that is closely linked to the work at Shimizu.

Apart from Matsunaga, two other leading academics are spear heading the efforts to develop biological techniques for absorbing carbon dioxide. Shigetoh Miyachi, former director general of the Institute of Applied Microbiology at Tokyo University is executive director for research at the Shimizu and Kamaishi laboratories. And Isao Karube of the Research Center for Advanced Science and Technology (RCAST) at Tokyo University is a leader of RITE's effort.

They all know each other very well, says Matsunaga, and they all sit on committees judging each others proposals, so it is easy for them to coordinate their research to complement rather than compete with each other. One aspect of the collaborations that may seem unusual to Western eyes is the involvement of banks, trading companies and life insurance firms. These firms do not have any say in the research, Miyachi says.

There are, however, several other reasons for such businesses to participate, says Katsuhiko Umehara, senior deputy director of MITI's Agency of Industrial Science and Technology (AIST). Japan's huge trading companies are very diverse and have technology departments that are always on the lookout for the "seeds of new technology and new business". Banks are "very eagerly seeking new customers" and hope to establish business with the new semi-private research organizations. Finally, he says, there is an element of "*matsuri no kifu*" — a Japanese phrase that translates roughly as a "donation to the village festival", implying that the companies contribute for no reason other than the wellbeing of the community.

It was, of course, just this type of long-term thinking that helped Japanese industry to dominate the rest of the world in selling such things as video cameras and home stereos. The Japanese are hoping to repeat their success in the coming market for technology to keep the world green.

David Swinbanks.

Algae to the rescue

Koganei & Shimizu

TADASHI Matsunaga and Shigetoh Miyachi, two of Japan's leading microbiologists, believe that tiny, genetically engineered photosynthetic microorganisms may help to solve worries about global warming by mopping up carbon dioxide emitted from power stations and industrial plants. And Japanese industry and MITI are prepared to back their ideas.

Matsunaga, who heads the department of biotechnology at Tokyo University of Agriculture and Technology in Koganei in the outskirts of Tokyo has built a



2-litre prototype 'biosolar reactor' that can absorb all the carbon dioxide out of ordinary air bubbled through the reactor at 300 millilitres per minute. The reactor contains a genetically engineered marine cyanobacterium, *Synechococcus* sp., and is filled with a stack of 600 light-diffusing optical fibre cables that ensure even lighting and optimum growth of the bacterium throughout the vessel.

The optical fibre cables, which, unlike conventional optical fibres, emit light of constant intensity laterally as well as along their length, were developed by a two-man Japanese venture company Science and Technology International and are a vital component of the bioreactor. Previous attempts by Matsunaga's team to absorb significant amounts of carbon dioxide were confounded by the fact that green-coloured photosynthetic microorganisms rapidly attenuate incident light and only those within a few millimetres of the light source can grow efficiently. The stack of closely packed optical fibres overcomes this problem by providing a constant lateral glow. In an industrial-scale biosolar reactor sunlight would be fed into the fibres (hence the name biosolar).

Matsunaga's reactor, however, is still a long way from providing a practical means of absorbing carbon-dioxide emissions from power plants and industry because the levels of carbon dioxide in such emissions are much higher than the 0.03 per cent in air. And high levels of carbon dioxide usually inhibit growth of photosynthetic microorganisms.

But researchers working under Miyachi, who heads MIT's marine biotechnology laboratories in Shimizu and Kamaishi, may have found a solution. They recently isolated a strain of marine

green algae off the coast of Kamaishi that grows happily at concentrations of up to 20 per cent carbon dioxide. By isolating the genes responsible for carbon dioxide tolerance, they hope to genetically engineer strains of microorganisms that can efficiently assimilate carbon dioxide at high concentrations.

Even so, it would require an enormous biosolar reactor to cope with the output of a power plant. Matsunaga says a typical megawatt-class power plant emits about 200 tons of carbon dioxide per hour. And if the biosolar reactor absorbed only a few per cent of this it would produce several tons of algal sludge per hour.

Matsunaga and Miyachi differ on how to deal with the sludge. Miyachi hopes to create strains of calcareous algae that can mop up the carbon dioxide and convert it into calcium carbonate that can be dumped at sea. His laboratories have been searching for suitable strains of calcareous algae in coral reefs in Palau and the Great Barrier reef off Australia.

Matsunaga, on the other hand, is trying to create strains of photosynthetic microorganisms that produce useful extracellular products that are released into the green brew of the reactor. For example, the *Synechococcus* in Matsunaga's prototype reactor has been genetically engineered to produce the amino acid glutamate. And his team have other strains that produce antibiotics and plant growth hormones.

A remarkable feature of the research by the two groups is the enthusiastic backing it is getting from industry. Matsunaga's research is supported by six companies, including a cement manufacturer, Onoda Cement, and Pentel, a maker of ball-point pens, through a new cooperative research centre at Matsunaga's university that was established by the Ministry of Education, Science and Culture last April to encourage collaboration with industry.

The education ministry and industry are providing him with ¥30 million each in fiscal year 1991 to build a 30-litre biosolar reactor and other facilities. And Matsunaga hopes to bring the total up to ¥100 million (\$750,000) a year with other donations from industry. He has a team of 30-40 researchers, about a quarter of whom come from the supporting companies.

Similarly, Miyachi's laboratories are staffed almost entirely with people from industry and the laboratories receive substantial support from MITI and private companies. Most of the researchers are beginners in biotechnology and marine science but Miyachi boasts that these 'brave laymen' isolated the strain of marine algae tolerant to carbon dioxide that might one day help solve the global warming problem.

D.S.

Germany in perspective

SIR — Your pen-lashing of Mr Nicholas Ridley (*Nature* 346, 203; 1990) was adequately rebutted by W. Frank Harris and J. F. Mackenzie (*Nature* 347, 510; 1990).

Whether the fears and misgivings of German characteristics are justified or not is beside the point. The point is that fears about the long-term fall-out from German reunification are real and are by no means felt only in Britain. As a cardiologist, I have no expertise in genetics, but I do know that recent German policy on 'eugenics' killed my parents in the gas chambers.

I have been prompted to react only after reading the self-justification of German physician-scientist E. Guthy (*Nature* 348, 670 1990). According to Guthy's criteria, I am qualified to articulate my ideas of "national or racial characteristics" having been exposed to a foreign culture and country from 10 May 1940 to 5 April 1945. That was quite an exposure. During those five years, Dutch culture vanished, all traffic signs and death warrants were written in German, 100,000 Jews were *ausgeradiert*, innocent people were executed without trial, our country was plundered and its infrastructure destroyed; and everything, indeed, done with a "striving for perfection and overdoing things" unparalleled in human history. Germany had outdone itself.

Nevertheless I believe that our — indeed my — hatred should come to an end and this has actually happened. It is only temporarily rekindled by discussions like the present one. But the fear, one hopes totally unjustified, persists.

Although Guthy acknowledges "the destruction and suffering inflicted upon other peoples by Germans in this century", he does not seem to be sufficiently aware that the ideas of Nazi ideology were written in the same language that he uses at home. What he euphemistically calls "Nazi ideology" was a German ideology. Far from being an anomaly, it was very much part of Germany: most Nazis were German and most Germans were Nazi.

On 9 November 1989 (the anniversary of *Kristallnacht*), when the Berlin Wall came down, we saw on live television moderate German leaders bragging about the reunited Germany and how proud (not humble) they were about their new 'Gross-Deutschland'. No word was uttered about why the partition of Germany had taken place 44 years earlier. It will take quite a few generations before the Riddleys and the Meijlers fully trust Germany again.

In itself, Guthy's letter is not a bad one; in principle, he may even be correct. The problem is, that it was written by a German, and that Germany's recent history is too bleak for German intellectuals to face. German history can never be undone or outdone, not even by the histories of the Portuguese, Spanish, British, Dutch and French. I wish

that the international scientific community could help its German colleagues with this problem. But it is discouraging if present-day German scientists still seem not to understand the emotions of the victims of their parents.

FRIITS L. MEIJLER

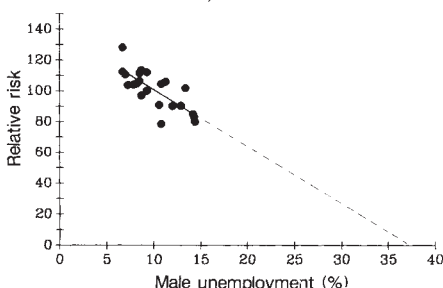
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Leukaemia and unemployment

SIR — The causes of cancer are mysterious indeed. There is a remarkable negative correlation ($r = -0.77$, $P = 0$) between risk of leukaemia and male unemployment by county in England and Wales (see figure). Extrapolating wildly through the data points towards the x-axis we see that cancer risk falls to zero when male unemployment reaches 37 per cent. Whatever the basis of this association (presumably some function of economic prosperity) it is clear that Her Majesty's Government has a coherent strategy to tackle the national leukaemia problem.

SIMON P. WOLF

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Incidence of leukaemia/lymphoma (age- and sex-standardized relative risks for 22 counties and 14,850 total cases of lymphoproliferative disease in England and Wales collated from 1985 to 1988) versus male unemployment at time of 1981 Census. Data were obtained from (1) *Leukaemia and Lymphoma: an Atlas of Distribution within Areas of England and Wales 1984-1988* (R. A. Cartwright, F. E. Alexander, P. A. McKinney, T. J. Rickets, London, Leukaemia Research Fund, 1990) and (2) Office of Population Censuses and Surveys, *Census 1981, Key Statistics for Local Authorities*, HMSO, 1984.

Gene manipulation

SIR — I wish to raise an issue of potentially great importance to programmes for the manipulation of human genes.

Conception is a matter of vital importance, and is probably the outcome of several

factors evolved in the search for the most suitable offspring. These factors are at present poorly understood. Much more experimental work will be necessary before it will be possible to identify the ethical principles that should govern such work.

But what if our capacity to determine ethical questions — morality in short — is itself linked with our genes?

Only if that surmise is proved wrong could a research venture boast that it had been conducted ethically. This question in my opinion should be widely and openly discussed.

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Time of grace

SIR — As a matter of course, I regularly scan the classified advertisements at the back of *Nature* in the hope of one day seeing a position that is tailor-made for me. I note, however, that while advertisements from overseas institutions have closing dates generally a month after the publication date, these from institutions in the United Kingdom average two weeks. This means that by the time the issue reaches the libraries in these remote corners of the globe, one generally has less than five days to make an application and get it to the United Kingdom. I'm sure its not a planned conspiracy to make sure researchers currently overseas cannot apply for such positions but wish to suggest a little a little more thought in this regard. *Nature* has an international readership.

SIMON MALPAS

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Lacuna seeker

SIR — I recently took a 'straw poll' of a class of 83 first-year biology students, to see how many of them had come upon the notion that, even in these banalistic times, there is such a thing as the philosophy of science, and that reductionism constitutes one approach by which one may seek to understand nature. To this end I asked them if they had come upon the term 'reductionism'. Not one of them had.

May I invite other academics to carry out a similar exercise? The results might persuade them that knowledge among science undergraduates of even the most basic philosophical ideas constitutes a lacuna which merits removal.

DOUGLAS B. KELL

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Greek tragedy moves on one act

Quite why Dr David Baltimore, now president of the Rockefeller University, should so vigorously have defended a disputed 1986 paper of which he was a co-author has never been self-evident. Now the paper has been retracted.

THE long-running dispute over Dr David Baltimore's involvement with a research article on the use of the transgenic mouse in the study of the genes of the human immune system seemed, two years ago, to have all the makings of a Greek tragedy (*Nature* 333, 795; 1988). And so, now, it has emerged. In Euripides, the moving tragedies are those in which a concatenation of misfortune is needlessly threaded through the patterns of people's lives not by intractable external circumstances, but because of the implacable influence of some person's human failing, pride perhaps.

The circumstances of this case are nevertheless both memorable and significant (see page 262), and will cast a long shadow over the conduct and even the reputation of research, not least because of Baltimore's high personal reputation and the respect which that commands. Baltimore was still only 32 when, in 1970, he announced (independently of but simultaneously with Dr Howard Temin) the discovery of reverse transcriptase, the enzyme that converts genetic information embodied in an RNA sequence into an equivalent sequence of DNA (*Nature* 226, 1,209; 1970). It was inevitable that, in 1975, Baltimore and Temin (and Dulbecco) should have shared the Nobel Prize for Physiology and Medicine.

Baltimore's achievements since then have been remarkable, both as a scientist and administratively. Among many other things, he was among the first to recognize the importance of transgenic mice, animals grown from embryos into which foreign genes have been transplanted, as a means of studying the functions of human genes. The transgenic mice used in the disputed and now retracted article (Weaver, D. *et al.* *Cell* 45, 247; 1986) came from a line founded two years earlier by Weaver (at Columbia University) and Baltimore.

Baltimore's influence as an administrator has also been considerable. For the past decade (since 1982), he has been best known as the first director of the Whitehead Institute at the Massachusetts Institute of Technology (MIT), the institution endowed with a benefaction of more than \$100 million of 1980 money derived from the sale of the cosmetics company Revlon. There are many who believe that the endowment would not have been made had Baltimore not been available as the founding director.

Baltimore has never been a powerbroker in the most familiar US pattern, which entails commuting to Washington to run powerful committees, but he was the co-chairman

(with Samuel O. Thier, president of the US Institute of Medicine), of the important (and liberal) report (in 1984) on the AIDS epidemic. It was only natural that, last year, he should have become the president of the Rockefeller Institute in New York, where he had been a PhD student from 1960 to 1964.

There is no reason why a single retracted article should interrupt these plans, but the protracted antecedents of the affair will cast a cloud on them. The case is amply documented. The first whistleblower was Dr Margot O'Toole, an untenured researcher in Dr Thereza Imanishi-Kari's laboratory, who alleged that she had been unable to repeat measurements reported in the disputed paper and that she had subsequently been unable to verify that the data published had actually been obtained in the laboratory.

O'Toole appears first to have taken her complaints to Baltimore, which may be considered to have been a proper course of action. Baltimore has said that he heard her out courteously, explaining why she was mistaken. O'Toole's account is that he dismissed her version of events peremptorily. Those who know Baltimore's emphatic way of making a case he believes to be correct will readily acknowledge that the two accounts can be reconciled. O'Toole's complaints were investigated internally at MIT and by a panel appointed by Tufts University, resulting in opinions matching that of Baltimore.

Part of the damage done by the affair is that suffered by O'Toole, whose temporary employment was terminated by Dr Imanishi-Kari. (After an interval of some three years, O'Toole is now employed at the Genetics Institute, the Boston-based biotechnology company.) It appears, nevertheless, that the only respect in which her conduct can be criticized is that she took with her 17 photocopied pages of Imanishi-Kari's laboratory notebook, and turned them over to Mr Walter Stewart and Dr Ned Feder, the two National Institutes of Health (NIH) researchers with a reputation for conducting zealous investigations of impropriety in research.

Baltimore was well within his rights in scorning the request from Feder and Stewart for access to the experimental data, but the haughtiness of his rejection of it may have helped to precipitate the NIH investigation that followed. It may be of some interest that, in 1987 and 1988, *Nature* declined to publish two versions of a manuscript in which Feder and Stewart spelt out what they considered to be errors in the published paper, partly because it then seemed probable that

the matter would be properly investigated. In retrospect, their arguments are more appealing than they may then have seemed.

Baltimore has often spoken as if he believed that Feder and Stewart had a personal animus against him, and as if that were generously reciprocated, especially against Stewart, about whom he has been colourfully eloquent, as only one who is convinced that he must be right can be. In the event, Stewart was seconded by NIH to the congressional Dingell committee, which announced its intention to investigate the disputed paper.

Why did Baltimore feel so strongly? On the face of things, there was no reason why he should have been so concerned. While Imanishi-Kari's study was potentially interesting, in that it showed that artificially introduced human transgenes could determine the idiosyncrasy of the immunoglobulin molecules produced by transgenic mice, many of those working in the field were at the time persuaded that the results, although suggestive, would require further investigation (which is not an unusual circumstance). Baltimore himself acknowledged that he did not follow Imanishi-Kari's view that the data provide support for Jerne's network theory, but that the data rather than their interpretation were his sole concern.

It would have been entirely possible, but also legitimate, for Baltimore to have been much more detached from the affair. He had arranged for the transgenic mice, and had helped to plan and write up the study, but the experimental data had been gathered in Imanishi-Kari's laboratory. Why take the risk of vouching for their accuracy? Some of Baltimore's friends (this one in particular) urged him to make some kind of public statement shedding full personal responsibility for the study. But they revealed an angrily defensive person, most offended that work with which he had been associated should be challenged.

Baltimore is by no means the first, and will almost certainly not be the last, distinguished researcher to be taken in by the errors (now alleged to be falsifications) of a close colleague. His defence of himself and Imanishi-Kari had been conducted with aplomb, resting on the basis that scientific freedom is threatened by an excess of zealous inquiry from persons outside the community and from the Congress. Especially memorable are his "Dear colleague..." letter, widely circulated in 1988, and his indignant riposte to Congressman John Dingell at the end of the public hearings on the subject in 1989.

John Maddox

Raised to a higher plane

Francis C. Moon

ONE of the more popular tricks to play with the new high-temperature superconductors is to levitate a magnet over a sample cooled in liquid nitrogen. Indeed, researchers at one Japanese laboratory, having used the levitation force to raise a goldfish in its fish bowl (reported in *News and Views* last year¹), have now proceeded to raise their director in the same way (see Fig. 1). For practical purposes, however, the important parameter is the stiffness of the force, both vertically and laterally. New work by Johansen *et al.*² at the University of Oslo shows that the high-temperature superconductors are much more stable laterally than had been predicted theoretically.

As is often the case with new discoveries, such as the high-temperature superconductors, the most obvious applications are not always the first to have the greatest industrial impact. This may become the case with the emerging field of superconducting magnetic bearings. With practical, high-current, wire-

wound, high-temperature devices still years away, some laboratories are beginning to look at levitation applications using new bulk superconducting materials. Although not as glamorous in the public's mind as, say, magnetic resonance imaging, these applications include passive, magnetically levitated (and hence friction-free) machine elements such as high-speed rotors (Fig. 2) for gyros, energy or momentum storage, cryopumps, cryocoolers and even high-speed machine tools. New discoveries in materials processing of bulk superconducting materials, especially the melt-quenched or melt-textured yttrium-barium-copper oxide (YBCO), have led to increased research on the nature of magnetic levitation forces between superconductors and permanent magnets.

Although countless laboratories have used levitation of small magnets (usually of the high-field rare earth element type) to demonstrate the phenomenon of superconductivity, very few have actually measured the forces and stability characteristics of these magnetic forces. For a levitated rotor made of a cylindrical rare earth magnet to be stable, it should have positive magnetic stiffness in five senses: one for vertical heave, two for lateral motions, and two more for pitch and yaw stability. In our experiments of 1988, we found³ that the equivalent magnetic pressure on the face of the levitation magnets produced by sintered YBCO was quite low, of the order of 0.2 newtons (N) cm⁻². Currently available gas bearings provide a much higher fluid pressure of the order of 5–15 N cm⁻². Progress in the past two years in the processing of YBCO using so-called melt-quench or melt-textured techniques have pushed the levitation pressures up to 5 N cm⁻² at 78 K and more than 10–20 N cm⁻² at 20 K. It was these developments that permitted the Japanese workers at the ISTEK institute to levitate their director on a floating platform using 250 samples of melt-quenched processed YBCO. However, engineers say that bearing stiffness is often the critical factor for many applications – thus the shift in focus in superconducting bearings from lift to magnetic stiffness, particularly lateral stiffness.

The Oslo group now show that, for a flat superconductor, the lateral stiffness seems to be independent of the position. A small lateral displacement of a test magnet yields a restoring force which is believed to be related to the pinning of magnetic flux lines to features in the superconductor. Davis has developed a model⁴ for calculating this stiffness using Bean's critical-state model⁵ in which the flux penetration into the superconductor is determined by the critical current of the material. The model predicts correctly the qualitative behaviour of the lateral force as seen by Johansen *et al.* But the model pre-

dicts a magnetic stiffness that is at least 50 per cent too small. Independent observations⁶ show that the vertical stiffness depends on the incremental displacement: the stiffness for small displacements of a few micrometres is as much as twice that for displacement of 100–1,000 μm . The Oslo measurements were made with displacements of 500–1,000 μm .

Johansen *et al.* also find a lateral magnetic drag force when the displacement exceeds



FIG. 2 Frictionless bearings could be an early use of high-temperature superconductors. Here, a half-kilogram magnetic disk suspended over three melt-quenched YBCO pellets is spinning 15,000 times a minute.

1–2 mm. This force might be interpreted as breaking the flux pinning forces when the test magnet is moved too far relative to the surface of the superconductor. In practical superconducting magnetic bearings, these drag forces can provide damping for lateral motions of the rotor. My colleagues and I find⁷ that the lift force and magnetic stiffness are related by a power law. A similar law was derived for this magnetic drag force which also relates magnetic lift and drag by a power law. Since the lift force is proportional to the magnetic field, increasing the applied field of the levitation magnet will also increase magnetic stiffness and drag. Quadrupole and multipole arrangements for the levitating magnets also increase the magnetic stiffness (W.C. Chu, personal communication).

Although wire and thin-film research still garner the bulk of the superconducting research funds, NASA is taking a special interest in magnetic levitation, looking at applications for machines which require long-life bearings (for use on missions to Mars and the Moon, for example) and have natural cryogenic environments such as liquid H₂ and O₂ propellants. Given the potential for high-speed, contactless, no-wear, stable levitation of small and large rotors, superconducting magnetic bearings may become the first 'sleeper' application of the high-temperature revolution. □

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FIG. 1 Levity in superconductivity research: S. Tanaka, director of the Superconductivity Research Laboratory at ISTEK in Tokyo, raised a centimetre above a superconductor at a recent meeting. The total mass of Tanaka and the magnetic disk supporting him was 120 kg.

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LTP is a long term problem

Frances Edwards

Is long-term potentiation of synaptic transmission in the brain a change in release from the presynaptic terminal or a change in the postsynaptic response? Last year, two groups applied classical methods of quantal analysis to address this question^{1,2} and concluded that the origin of the change is presynaptic. But in a paper that appears on page 344 of this issue³, Larkman *et al.* question whether this analysis is appropriate.

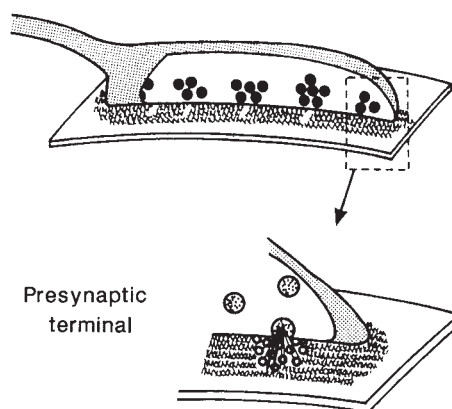
During the 1950s the elegant work of del Castillo and Katz⁴, Boyd and Martin⁵ and others laid the basis of quantal analysis and of our present knowledge of synaptic transmission. From these early investigations of transmitter release, and the extensive studies over the following two decades, synaptic transmission at the neuromuscular junction became one of the most thoroughly characterized and best understood of physiological phenomena.

Quantization of amplitudes of synaptic signals at the neuromuscular junction has long been accepted to be a presynaptic phenomenon. Discrete packets of acetylcholine are thought to be released from the presynaptic terminal onto an effectively unlimited spread of receptors on the postsynaptic membrane opposing the release site (*a* in the figure). Statistics developed from this classical work make it possible to find out whether changes in postsynaptic response at the neuromuscular junction have their origins pre- or postsynaptically (see box).

Analysis of transmission at synapses in the brain has been more difficult because the signals to be measured are much smaller than at peripheral synapses and the neurons are less accessible. Work on the excitatory synapses on hippocampal CA1 pyramidal cells now presented by Larkman *et al.*³ addresses the

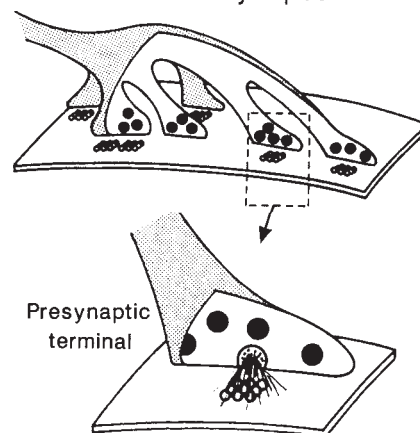
question of whether the mechanism of synaptic transmission is the same in the central nervous system as in the periphery. Their report, and others dealing with this and other

a Neuromuscular junction



Unlimited postsynaptic receptors

b Central synapse



Postsynaptic quantal group

Schematic diagrams of models of synaptic transmission. *a*, The neuromuscular junction features a large presynaptic (nerve) terminal with multiple release sites opposed to a postsynaptic (muscle) membrane densely covered with receptor/channel molecules. The inset represents the opening of some of the channels (o) in response to release of transmitter from a single vesicle (for simplicity, folding of the muscle membrane is not shown). *b*, A central synapse consists of many boutons, each behaving in an 'all or none' manner⁶. Thus release of a single vesicle of transmitter will saturate all receptors opposing that bouton (inset). The groups of circles represent hypothesized quantal groups of receptors. Note that the membrane opposing a bouton may contain a variable number of quantal groups.

synapses^{6,7}, show that central transmission is quantal but that, unlike the neuromuscular junction, the quantization at synapses in the central nervous system is probably determined by the availability of postsynaptic receptors (*b* in the figure).

For some time now, studies of central synaptic transmission have been dominated by the phenomenon of long-term potentiation.

of controversy. It is not clear, for example, whether it is a pre- or postsynaptic phenomenon, or a combination of both. Taking advantage of technical advances⁸, two laboratories^{1,2} have made high-resolution recordings of synaptic currents and, following the neuromuscular model of quantal synaptic transmission (see box), have fitted their data with simple binomial distributions. The authors estimated numbers of failures and changes in the variables n and/or p , before and after induction of long-term potentiation and applied the assumptions used in quantal analysis of neuromuscular transmission. Both groups concluded that the changes measured were presynaptic in origin.

In the light of the results obtained by Larkman *et al.*, and those from previous work on central synaptic transmission⁶, fitting the data with a simple binomial distribution is not justified. Moreover, the observation of skewed and in some cases multiquantal miniature distributions in the central nervous system, and the hypothesis of a graded synaptic response along dendrites⁹, suggest that even the assumption of identical postsynaptic response at all release sites is by no means safe. Without these assumptions the interpretation of the origin of changes in the variables n and p and related statistics is certainly open to question.

According to a hypothesis of the mechan-

Quantal analysis at the neuromuscular junction

At the neuromuscular junction, synaptic events can generally be well resolved above the background noise. Amplitude distributions of miniature (single-quantum) currents can be fitted with a single gaussian distribution with a mean (q) reflecting the quantal size and variance (V) around the mean. Amplitude distributions of evoked end-plate currents or end-plate potentials fall into several equidistant peaks which can be fitted on the assumption that the peaks consist of integer multiples of the amplitude distribution of the miniature currents. Such a distribution can be described by binomial statistics as a function of the measured parameters q and V , and can be used to estimate the parameters n and p : the relative number of events falling into each peak depends on p , which is thought to reflect the probability of release from each release site, while n is thought to reflect the total number of release sites. Even when individual peaks are not resolvable, a binomial relation can be fitted to the distribution and some of the variables estimated. Binomial analysis of the amplitude distributions recorded at peripheral synapses has allowed comparison of different synapses and investigation of plasticity of particular synapses based on attempts to interpret the experimentally determined values of q , V , n and p . Although, even in the periphery, the interpretation of n and p has been a matter of debate, such analysis has been used to determine whether changes in amplitude distributions of postsynaptic events have a pre- or postsynaptic origin. This interpretation necessitated various assumptions, for example that the response to release of a single vesicle from any release site has the same distribution (as appears to be the case at the neuromuscular junction).

isms of central transmission, put forward by myself and colleagues⁷ (*b* in the figure), receptors may be grouped quantally in the postsynaptic membrane. We suggest that the membrane opposing a single bouton may contain a variable number (0 to about 4) of these quantal groups, all of which are saturated by release of a single vesicle of transmitter. Such a hypothesis puts in doubt even the interpretation of failures, as release of a vesicle may not produce a postsynaptic response.

Before the question of the pre- or postsynaptic origin of long-term potentiation can be resolved, it seems the details of signal transmission in the central nervous system must be clarified. Indeed, even when a clear model is established, as Larkman *et al.* sug-

gest, "a more complete quantal analysis will be required to quantify the relative importance of pre- and postsynaptic changes". □

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COSMOLOGY

A quasar superstructure

Joseph Silk and David Weinberg

THERE have been occasional reports over the past decade of groupings of quasars, the most luminous and most distant objects in the Universe. Clowes and Campusano, reporting in the latest *Monthly Notices of the Royal Astronomical Society*¹, now present evidence of an elongated grouping, which may both exceed in scale and be far more distant than any known large structure of galaxies.

Clowes and Campusano obtained their data in an automated search of a field of some 25 square degrees, in an area where earlier investigations had suggested an anomaly in the quasar redshift (distance) distribution. An objective prism used with the UK Schmidt telescope allowed low-resolution spectra of many objects to be obtained over a wide area. Quasar candidates were distinguished by a computerized search that looked at both emission lines and excess ultraviolet light. Follow-up spectra were obtained to confirm the quasar identifications and obtain precise redshifts. A total of about 60 quasars were discovered in the field.

By inspecting the redshift distribution the authors found ten quasars with redshifts between 1.2 and 1.4 (about a quarter of the distance to the most distant known objects). Although there were other peaks in the distribution, statistical analysis suggested that only these ten quasars form a grouping that is likely to be physically associated. The group is elongated, presenting a profile about 1° by 2.5–5°. Its depth probably exceeds 100 *h*⁻¹ megaparsecs (the exact size depends on the value of Hubble's constant, normally given as 100 *km s*⁻¹ *Mpc*⁻¹, with 0.5 ≤ *h* ≤ 1). Clowes and Campusano found unambiguous evidence for clumping in the field over 1° (or 35 *h*⁻¹ *Mpc*), and more tentative evidence for clustering over 5° (in particular, nine of the ten quasars lie above the main diagonal of the survey plate).

There have been several claims for very

large structures in the local distribution of galaxies and galaxy clusters. These include the 150 *h*⁻¹ *Mpc* 'Great Wall' revealed in the Harvard-Smithsonian Center for Astrophysics redshift survey², the 300 *h*⁻¹ *Mpc* supercluster complexes identified by Tully^{3,4} in the Abell cluster catalogue, and the signals of 120 *h*⁻¹ *Mpc* periodicity observed by Broadhurst *et al.* in deep redshift surveys through the galactic poles⁵. The challenge in such cases is to evaluate the statistical significance of the giant structures, especially because galaxies and clusters are already known to cluster strongly on smaller scales. Postman *et al.*⁶ argue that Tully's results can be explained in terms of the known cluster correlations on scales of about 30 *h*⁻¹ *Mpc*; and Kaiser and Peacock⁷ and Park and Gott⁸ show that signatures of 'periodicity' can arise in pencil-beam surveys much like that of Broadhurst *et al.* as a result of small-scale galaxy clustering, without any special predisposition towards 120 *h*⁻¹ *Mpc* structure in the galaxy distribution.

Similarly, although the cold dark matter (CDM) model generates structure only weakly at 150 *h*⁻¹ *Mpc*, Park⁹ and Gunn and one of us (D.W.)^{10,11} find that structures such as the Great Wall arise in numerical simulations of CDM as occasional, chance alignments of three or four smaller superclusters. But statistical studies of the galaxy angular correlation function¹² and the variances of the galaxy density field revealed by the Infrared Astronomical Satellite (IRAS)^{13,14}, provide clear evidence of an excess of large-scale clustering over that predicted by the standard CDM model.

For the quasar survey by Clowes and Campusano¹, one must ask whether the 100–200 *h*⁻¹ *Mpc* structure is statistically significant, or whether it consists of smaller groups and chance outliers linked by a 'join-the-dots' philosophy. The question is difficult to answer with the current data because

the larger scale is close to the angular size of the survey plate itself. An earlier study of a different field by Crampton *et al.*¹⁵ found a grouping of 23 quasars at a redshift of 1.1, with an inferred physical scale of about 60 *h*⁻¹ *Mpc*. Such large quasar groups seem to be rare, because they have not been reported in other faint, wide-angle surveys. Complete surveys of larger areas and greater depths should make it possible to assess the frequency and statistical significance of large quasar groups.

In contrast to the large galaxy structures, rare groupings of rare objects need not be associated with physical increases in the local mass density. If quasars formed from rare peaks of primordial density fluctuations that had random phases, one can readily show that they would have enhanced correlations¹⁶. The clustering of Abell clusters has been explained in this manner, and if there is typically one quasar formed per galaxy cluster then similar correlations would result for the quasar distribution. This 'biasing' mechanism might easily give strong quasar correlations out to scales of about 20 *h*⁻¹ *Mpc*, but it is difficult to account for 100 *h*⁻¹ *Mpc* structure in this way because one would not expect the underlying density fluctuations to be coherent over such large scales.

One cannot automatically dismiss the alternative possibility that the quasar groups represent physical enhancements in the underlying density of the Universe. This is the more radical view, which might be supported by the discovery of associated enhancements in the distribution of galaxies or X-ray-emitting gas. Such structures would be remarkable both for their total size and because, at a redshift of 1.3, they must already have formed when the Universe was less than half of its present age. They are not yet in conflict with other observations — the isotropy of the cosmic microwave background provides the closest constraint — but they would provide a further nail in the coffin of CDM, and they would probably challenge the traditional assumption of gaussian primordial density fluctuations. □

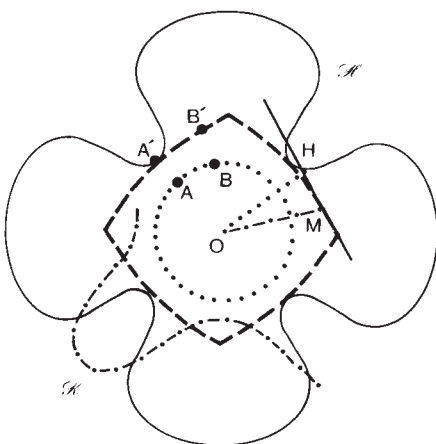
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The shape of crystals to come

J. Villain

THE most obvious property of solid objects is their shape. This shape is almost never an equilibrium property, but instead it depends on the history of the object. The case of crystals is of particular interest. Of fundamental interest, because it is associated with non-linear statics or dynamics. Of technological interest too — for semiconducting devices, for example. On page 322 of this issue¹, Berge *et al.* investigate the shapes that crystals can take when they grow two-dimensionally on the surface of water. Using the



A crystal with an arbitrary initial shape (dotted curve) tends to a faceted shape (dashed curve). The orientations of the facets correspond to the slowest growth rate. The full curve $\mathcal{H}$ indicates the Wulff construction for the growth shape. It is defined by $\mathbf{OH} = \mathbf{n}/v(\mathbf{n})$. The crystal surface is the envelope of the perpendicular in H to $\mathcal{H}$. A completely flat facet would be obtained if the full curve had a discontinuous slope in A. The dot-dashed curve $\mathcal{K}$ is generated by the vectors $\mathbf{OK} = \mathbf{n}/v(\mathbf{n})$. Frank proved that the points of the crystal surface where the normal $\mathbf{n}$ has a given orientation move on a straight line parallel to the normal to $\mathcal{H}$ at the corresponding point K, the point defined by $\mathbf{OK} = \mathbf{n}/v(\mathbf{n})$ for that precise value of $\mathbf{n}$.

surfactant sodium dodecyl sulphate (a soap molecule), they show that crystals can grow with curved and straight edges, and even with elaborate branched protrusions.

The true equilibrium shape of crystals can be reached in a reasonable time (and therefore be observed) in two cases only: in solid helium at equilibrium with the superfluid²; and in very small crystals³ (typically a few micrometres across). The equilibrium shape of an object is determined by the minimization of the surface energy for a fixed volume. This theoretical problem was solved in principle by Wulff at the beginning of this century, but interest has been renewed over the past 15 years. This is because a number of interesting features can arise from the various forms of the surface free-energy density $\sigma(\mathbf{n})$, a function of the local orientation $\mathbf{n}$

of the surface. Three-dimensional crystals generally (but not always) have flat facets and can have sharp edges, but need not.

One of the difficulties of predicting the forms is three-dimensional geometry. Two-dimensional crystals are easier. So far, these were an academic issue for textbooks and theorists. The experiments by Berge *et al.*¹ make two-dimensional crystals available, but, what the authors observe is not the final equilibrium shape, but rather the shape of growing crystals.

The simplest model one can imagine for describing the growth shapes of crystals is the following⁴: the growth rate v is assumed to be a function $v(\mathbf{n})$ of the local orientation $\mathbf{n}$ of the surface. Growth shapes exhibit facets⁵ if certain orientations have a particularly slow growth rate. For example, if the growth rate v is minimal at point A on the circumference of a growing crystal (see figure) and is much greater at point B, then as B progresses to B' and A to A', the line A'B' will eventually become parallel to the slow direction. So a facet parallel to the slow orientation is expected to form. Indeed, the growth rate can vary dramatically, so that growth shapes have often broader facets than equilibrium shapes⁵.

This model neglects all complications due to the conservation of energy and matter. For three-dimensional crystals, these laws can cause instabilities in the growth process⁶: minor changes in conditions can cause wild variations in the growth shape. They can also be responsible for the similar instabilities, such as the branched, filamentary growths, observed by Berge *et al.* One does expect the matter diffusing toward the growing crystal to reach the corners first and to give rise to elaborate patterns.

It is amusing to note that Berge *et al.* have also observed, at shorter times, the opposite effect, namely a rounding of the corners. As the effect is related to the concentration of molecules in solution, it is presumably also a result of matter conservation. These experiments cannot be interpreted in a quite standard way because although the patterns are two-dimensional, the dynamics that determine them can be three-dimensional, in that molecules will aggregate onto the crystal edges from the bulk solution.

Coming back to the simpler case of a crystal at equilibrium, the existence of flat facets (surfaces without steps) is just a consequence of steps having a positive free energy per unit

Lethargic viruses

POSTVIRAL fatigue syndrome, until recently viewed with great scepticism by the public and doctors alike, may itself be caused by a virus. (*Br. Med. J.* **302**, 692–696; 1991). Using the highly sensitive polymerase chain reaction, G.W. Gaw *et al.* detected enteroviral RNA sequences in 53 per cent of patients with the syndrome, but in only 15 per cent of control patients. The viral sequences were found in muscle tissue and the percentage might be higher if nervous tissue were to be investigated as well, because these are the two sites presumed to be affected in the disorder. Although the authors stress that the findings are preliminary, and do not constitute a diagnostic test as yet, the data are provocative enough to stimulate further hunts for viral agents in this distressing disease.

Oriental eons

CHINESE speech patterns and the pinyin transliteration systems make a furtive appearance on the pages of *Geology* this month (**19**, 195; 1991). The occasion is a plea on the Opinion page for a new unit of geological time, the year being ludicrously small and the second, the official SI unit, even more so. Writing in partial support of an earlier plea by H. Hofmann for the unit of a 'geon', equal to 100 million years, A.F. Trendall notes that Hofmann's quixotic alternative name for the unit — *i*, the pinyin rendition of the Chinese character representing $10,000 \times 10,000$ — could cause confusion if pronounced with the wrong inflection: with a falling tone, it would indicate the correct numeral, 10^8 , but with a high, flat tone it would represent the number 1. For the sake of completeness, Trendall points out the unit should be *yi nian*, for 10^8 years.

Molecular determinism

SOMEWHERE between small molecules and large biological structures one may expect that statistical properties, averaged over a large number of stochastic events, will give place to what G. Weber refers to as deterministic behaviour, caused by the presence in the system of measurably different populations. With L. Erijman, Weber has now obtained ingenious evidence that such a transition occurs in various subunit proteins between dimers and tetramers (*Biochemistry* **30**, 1595–1599; 1991). Pressure-induced shifts in the degree of self-association were measured and fluorescent labels allowed exchange of subunits in the quaternary structure to be followed. In dimers the two rates coincided, but in tetramers subunit exchange was two orders of magnitude slower. The tetramers are evidently a heterogeneous ensemble, in which the various individuals respond differently to the applied pressure and retain the imprint of their previous history for much longer than is needed for subunit dissociation.

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length. Indeed a step that crossed the whole surface of a three-dimensional crystal would have a huge energy and is forbidden. In a two-dimensional crystal, however, the surface is one-dimensional, a step on this surface is 'zero-dimensional', has a finite energy and is always allowed except at zero temperature. Thus a two-dimensional crystal cannot be faceted. In the pictures of Berge *et al.*, there are no really straight parts, and the impression of facets arises from the sharp corners. Sharp corners are allowed in two as in three dimensions.

The mathematics of crystal growth is a source of beautiful geometrical exercises. One approach is a transcription of the classical Wulff construction which gives the equilibrium shape. The surface free energy $\sigma(\mathbf{n})$ should merely be replaced by the growth rate

$v(\mathbf{n})$. However, this yields only the limiting shape at large times. More general theorems have been derived by Frank⁴.

After a talk delivered by Frank, Cabrera expressed his admiration as well as some disappointment because there was nothing left in the field. Since the work of Mullins and Sekerka we know that Frank only exhausted the simplest possible description of growth phenomena, and the experiments of Berge *et al.* illustrate the fact that this too simple picture breaks down after a finite time, when instabilities come into play, bringing dendrites and occasionally chaos. And that is a very modern field of investigation⁶. □

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GLOBAL WARMING

Breakup of Antarctic ice

H. Jay Zwally

WORDIE Ice Shelf, floating on the coast of the Antarctic Peninsula, has been slowly breaking up over the past few decades, according to Doake and Vaughan on page 328 of this issue¹. The observation, obtained from detailed analyses of satellite images, is of particular interest because the disintegration has occurred during a period of warming in the vicinity of the ice shelf, and because of growing concern about the fate of the entire Greenland and Antarctic Ice Sheets (grounded on the land masses) in a warmer climate. Because the ice shelves are important in restraining and, perhaps, stabilizing ice discharge from the continents, it is tempting² to see their breakup as a sign of impending doom.

How should we view the new result in the context of public concern about greenhouse warming, the potential melting of the polar ice caps, and estimates of rising sea level? Is it an indication of a serious trend or a solely regional phenomena? In particular, is the rapid change in area of the Wordie Ice Shelf relevant to the question of stability of the grounded West Antarctic Ice Sheet, described³ as "glaciology's grand unsolved problem"? Should this melt, the global sea level could rise by 5 metres.

Doake and Vaughan correctly note that although nearby ice shelves in the Antarctic Peninsula may be at risk if the warming continues, substantial additional warming would be required to affect the more-southerly major ice shelves that help stabilize⁴ the West Antarctic Ice Sheet. The breakup of the Wordie Ice Shelf (2,000 km² in area) is probably part of a regional phenomenon that is consistent with the regional warming in the Antarctic Peninsula, but it should not be extrapolated to other parts of Antarctica. In the vicinity, summer and autumn tempera-

tures have been rising at about 0.05 and 0.1 K a year, respectively, since 1958 although there has been no significant trend in winter and spring temperatures^{5,6}. Recent temperature trends in most regions of the Antarctic



Wordie Ice Shelf in 1966: waiting to disintegrate

are not significant, however, and sea-ice extent, which regionally follows temperature changes, has shown no significant overall trend⁵. And although large tabular icebergs (covering about 30,000 km² in total) have calved from the Ross and Filchner-Ronne Ice Shelves (which cover 1,000,000 km²) in recent years, such events are likely to be episodic and unrelated to the Wordie Ice Shelf breakup. Furthermore, ice shelves float in sea water, so that their breaking up into icebergs has no direct effect on sea level. In general, breakup of the Wordie Ice Shelf may be viewed as one of the many regional and global-scale changes that must be observed to discern the overall pattern of climate change, its causes and significance.

But the breakup of the Wordie Ice Shelf

does reveal something about the ice dynamics and the interactions between ice, atmosphere and ocean that determine the volume of polar ice and how it changes. Ice shelves are formed on the ocean as ice discharged from the continent coalesces, snow accumulates or ablates on the top surface of the shelf and ice melts or freezes to the bottom. They are especially vulnerable to climatic change because of their exposure to both oceanic and atmospheric warming. Observation of the breakup of an ice shelf provides vital information on the way the thickness of the ice shelf decreases and the way it fractures and disintegrates. Most of the ice discharging from the West Antarctic Ice Sheet does so in fast-moving ice streams that are at least partially restrained by the ice shelves. Therefore, the new result is directly relevant to the question of the future stability of the West Antarctic Ice Sheet.

The West Antarctic ice streams appear^{7,8} to be undergoing significant changes unrelated to any current climatic trends. But the enhanced melting of the restraining ice shelf and its consequent breakup provides an important mechanism for the irreversible unpinning of the West Antarctic Ice Sheet to proceed relatively quickly — over timescales of centuries⁴. Ice-shelf thinning and breakup could markedly accelerate the discharge of

grounded ice. However, it should be noted that the current climate of the major ice shelves is more than 10 K cooler than Wordie. Less consequential, but of more immediate concern, is the question of Antarctic mass balance (currently uncertain by around 30 per cent, equivalent to a change in global sea level of 2 mm a year). In contrast to the floating ice, the mass balance of the grounded ice responds very slowly to changes in temperature, but instantaneously to changes in precipitation that may accompany changes in temperature⁹. Continued observation of changes in the area of the Antarctic ice shelves and grounded ice sheet (with high-resolution satellite imagery) and the initiation of measurements of their volume change (by satellite laser altimetry) are urgently needed to determine the future growth or breakup of the Antarctic ice. □

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The earliest horsemen

Jared M. Diamond

HORSEBACK riding produced history's first revolution in land transport. Evidence from many parts of the world shows how riding transformed not only travel but also warfare, economies and social organization. Despite the importance of this watershed event, it has been very uncertain when and where riding originated. D. Anthony and D. Brown¹ have now identified bones of the earliest known ridden horse — they are from the Ukrainian steppes and date to around 4000 BC, 2,000 years before the earliest example identified previously. This particular place and time are of great interest because of their relevance to one of the most debated problems of historical linguistics, the origin of Indo-European languages.

Efficient control of a ridden horse depends on a bit which rests on soft gum between the incisors and premolars, and which lets a rider apply painful pressure to the horse's mouth. The first depiction of a ridden horse dates from about 2000 BC, the first preserved bit from about 1500 BC. But horses had already been domesticated for food by around 4000 BC in the Ukraine, where cracked horse bones at archaeological sites demonstrate that horses accounted for about half of the meat available to the Sredny Stog culture east of the Dnieper River. Circumstantial evidence, such as perforated antler tines similar to later cheekpieces for reins, had hinted that Sredny Stog horses might have been bitted and ridden as well as eaten.

Anthony and Brown's approach was to examine horse teeth for signs of wear produced by metal bits^{1,2}. Although bits are designed to rest on the gum, X-ray studies show that horses often use their tongue to lift the bit back onto the first premolar teeth, where the bit slides back and forth over the tooth's anterior edge. Hence Anthony and Brown examined first premolars from 10 modern domestic horses known to have worn metal bits and from 20 modern feral horses known not to have done so. Teeth of the bitted horses proved distinguishable to the naked eye because of wear (beveling) of the anterior edge by 2–8 mm (mean 3.6 mm), far greater than the 0–2 mm beveling (mean 0.8 mm) of feral horses. Scanning electron microscopy of first premolars from bitted horses further revealed a distinctive pattern of broken enamel over the entire front cusp.

Anthony and Brown then examined 19 horse first-premolars from archaeological sites dated at 2000–25000 BC in the Soviet Union, Iran and France. An exceptionally large seven- or eight-year-old stallion, buried together with two dogs, pottery and possible rein cheekpieces at the Sredny Stog site of Dereivka around 4000 BC, provided the earliest evidence of biting. The stallion's two first-premolars were bevelled by 3 and 4 mm respectively, and the whole biting sur-

face of the first cusps was densely covered with distinctive microscopic breaks indistinguishable from those produced by metal bits. Two first-premolars from the Iranian site of Malyan at 2000 BC also showed unequivocal marks of biting.

Why is this discovery so significant? Consider how riding transformed Indian society

Prancing power — horseman at the festival of Athene in Ancient Greece.

of the plains of both North and South America when escaped Spanish horses arrived long ahead of Europeans themselves³. Daily travel distance increased by many times to 80 miles a day, speed increased proportionally, and loads of 200 pounds per horse could be carried home. So one human group could cover a much larger territory, exploit distant resources and occupy environments that would previously have been uneconomic. Furthermore, riding transformed warfare long before the invention of stirrups and other techniques for fighting from horseback itself. Even bareback riders could stage surprise raids at will on distant communities and escape before reprisal, leading to the collapse of their unfortunate horseless neighbours. Finally, possession of valuable mounts created social divisions and concentrated political power within the horse-owners. These effects of riding are obvious not only in the Indian societies of North and South America after 1600 AD, but also in the transformation of warfare in the Near East when horses arrived around 2000 BC, the founding of equestrian kingdoms in west Africa, and the decisive role of a few dozen horses in enabling small bands of Spaniards to overthrow the Aztec and Inca empires.

These principles, illustrated by written records or recent events, are also reflected in at least three linked developments in pre-literate Eurasia 6,000 years ago. First, problems of distance and transport had previously made the treeless steppe of southern

Russia and central Asia uneconomic for human occupation. That changed dramatically when riding, followed by the invention of wheeled transport around 3300 BC, was added to the herding of sheep and cattle. From the Ukraine during the fourth millennium BC, steppe settlement, weapons and cultural traits rapidly spread for thousands of miles east nearly to Mongolia and west to the plains of Hungary.

Second, the appearance of the earliest horsemen is also reflected archaeologically in the collapse of the high civilizations of

southeast Europe^{3–5}. Just before 4000 BC, the area had supported large towns with two-storey houses, copper tools and ornaments, sophisticated pottery, multi-crop agriculture, and food storage. In the centuries following the burial of the Sredny Stog stallion, some of the towns grew to enormous defensive settlements and then collapsed, their ruins being replaced in the archaeological record by steppe weapons and other artefacts.

Finally, the advent of riding bears upon the long debate over the origin of Indo-European languages^{5–7}. The close similarity of so many languages, already distributed by Roman times from Britain to India, testifies to a large-scale linguistic replacement somewhat before the dawn of written history, by which a Proto-Indo-European language wiped out earlier European languages of which now only Basque survives. As to when and where the spread of the language began, a traditional view has it that word roots shared by surviving Indo-European language families include words for horses, other domestic animals and wheeled transport, but surprisingly few names of crops^{5,6}. That suggests that speakers of Proto-Indo-European lived in an area where agriculture and crop storage were much less important relative to herding than in the Near East or most of Europe. Hence speakers of the language are often taken to be the steppe invaders from the Ukraine in the fourth millennium BC. ►

But that view begs the question what cultural advantage permitted those invaders and their descendants to impose their language over such a vast area. The domestication of horses merely for food can hardly have been such a cultural watershed. With that in mind, Renfrew⁷ has persuasively argued that the earlier spread of farming itself to Europe was much more likely to have driven a linguistic expansion, as we know happened in the case of the expansion of the Bantu language in Africa and of the Austronesian language from southeast Asia. Yet that argument in turn fails to explain why reconstructed Proto-Indo-European has so few terms for crops compared to reconstructed Proto-Bantu and Proto-Austronesian.

Anthony and Brown's evidence for the invention of horseback riding in the Ukrainian steppe around 4000 BC is in striking agreement with the traditional model of the origin of the Indo-European language. The spread of Proto-Indo-European would have been powered not by riding alone, but rather by the addition of riding to pre-existing agri-

culture, herding and metallurgy, and by the subsequent invention of the wheel. Speakers of the language thereby became the first people to put together the economic and military package that dominated much of the world for the next 5,000 years. Through the accident of Proto-Indo-European speakers living at the right place and time, the language of these pages may be descended from that of the Sredny Stog horsemen, when it might otherwise have been related to Basque. □

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AIDS

Will therapy spread disease?

Robin A. Weiss

MIGHT the treatment of individuals infected with the human immunodeficiency virus (HIV) lead to an increase in the number of AIDS cases in society? That, in a nutshell, is what Anderson, Gupta and May argue on page 356 of this issue¹. Now that zidovudine (AZT) is being introduced for the treatment of HIV-positive subjects, but with no vaccine to prevent the spread of infection yet in sight, the longer life-span gained from drug treatment also provides an infectious person more opportunities to spread HIV to others. Anderson *et al.* show that when (1) HIV transmission rates are low, (2) treatment lengthens the incubation period before AIDS by only a few years and (3) the proportion of infected people undergoing treatment is small, then the total number of AIDS cases may increase as a result of therapy. Selective targeting of treatment to those with most sexual partners would make matters worse.

The circumstances in which therapy would increase AIDS deaths assume that drug treatment does not significantly reduce the infectiousness of the treated person, and that treatment and the counselling that should accompany it does not lead to 'safer' sexual behaviour or self-administration of narcotics. Of course, the evolution and spread of drug-resistant strains of HIV might also increase the overall load of AIDS.

What is at issue here is that medical treatments which benefit the individual might harm the overall well-being of the community. Anderson *et al.* point out that the principle is not novel, citing their own recent study of rubella in situations where vaccine cover is incomplete. One may also surmise

that the simple and effective treatment of sexually transmitted bacterial infections such as gonorrhoea and syphilis by antibiotics encouraged a more permissive attitude to engaging multiple sexual partners, especially among gay men, just at the time when hepatitis B virus and as yet unknown HIV was spreading in that community. Anderson *et al.* point out that "it is unethical to refuse treatment to individuals, given that AIDS is lethal and that zidovudine might slow progression to AIDS". They hope, therefore, that their study will draw further attention to the need to improve counselling aimed at high risk behaviour.

It is difficult to judge how important the type of mathematical modelling of HIV transmission carried out in this study really is. To my mind, the authors' most telling comment is that their analysis emphasizes the need to assess the degree to which antiviral therapy suppresses infectiousness. There is so little knowledge about infectiousness and modes of HIV transmission from infected individuals that the premises on which the models are built must be speculative. The homosexuals on which the modelling is based have, by and large, responded remarkably in their sexual practices to counselling or maybe to bereavement. I am not aware of any data on HIV transmission from treated individuals being treated with zidovudine. And the evidence is by no means clear that p24 antigenaemia (the amount of viral core antigen) correlates with infectious HIV titres in the blood^{2,3}, let alone with infectiousness by the sexual route.

If we are to determine the infectiousness of

HIV-positive individuals, we need to be more precise about the concepts of transmission, treatment and prevention, as well as more diligent in gathering epidemiological and microbiological data. I would question the assumptions by Anderson *et al.* that receptor decoys⁴ represent immunotherapy, and, more important, that immunotherapy is equivalent to vaccination. We do not know whether HIV 'carriers' are chronically or intermittently infectious, or whether sexual transmission is mainly through cell-free virus or infected monocytes and lymphocytes, blood, or seminal and vaginal fluid. The heterosexual spread in Africa points strongly to concurrent venereal infections, particularly chancroid and herpes simplex sores, as important co-factors in HIV transmissions⁵. The role of such cofactors in the spread of HIV and progression to disease will be important in further modelling of the AIDS epidemic, as Anderson *et al.* noted previously⁶.

An article in last month's *Scientific American* on sexually transmitted diseases in the AIDS era makes compelling reading. Aral and Homes⁷ depict how HIV infection, alongside other venereal infections, is growing fastest in the United States among urban minority populations when social disintegration, prostitution and sex in exchange for drugs hasten the spread of AIDS. Thus the epidemic of the Third World in our midst is coming to resemble that in developing countries. In the United States, AIDS has become the leading cause of death among men aged 25-44 years (bar 'unintentional injuries') and will soon be so for women too⁸.

When we do have long-term anti-viral drugs, and efficacious vaccines that actually prevent HIV infection, those covered by medical insurance will no doubt benefit from them greatly. To what extent they will impinge on the demography of the AIDS pandemic is another matter. On a visit last year to one of the poorest countries in Africa, some colleagues and I noted that health education posters about AIDS were much in evidence at the regional hospital, exhorting people to use condoms. But the pharmacy was bare. The hospital superintendent proudly showed us the acupuncture clinic where the used needles were bathed neither in alcohol nor in hypochlorite. Polio and measles take a significant toll of infants since there is still no vaccination available. In that setting we need not fear that zidovudine will adversely affect the spread of AIDS. □

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Back to the chloride channel

Raymond A. Frizzell and William H. Cliff

THE product of the gene that is defective in cystic fibrosis may be a chloride channel. This is the implication of new work, reported by Anderson *et al.*¹ in *Science*¹ and by Kartner *et al.* in *Cell*², showing that expression of the cystic fibrosis gene in non-epithelial cells endows them with a plasma membrane Cl^- conductance that can be switched on by cyclic AMP. Activation of epithelial cell Cl^- channels by cAMP is widely recognized as the primary cellular defect in patients with cystic fibrosis. Because the authors demonstrate that such a conductance can be conferred on non-epithelial cells by expression of the cystic fibrosis gene product, they make

the simplest interpretation — that the protein itself is the Cl^- channel activated by cAMP.

The idea that the cystic fibrosis gene encodes a regulated Cl^- channel emerged when it was shown that Cl^- channels in cell-free membrane patches could be activated by protein kinases in normal cells but not in cells from cystic fibrosis patients (reviewed in ref. 3). A defect in the regulation of Cl^- secretion by cAMP had been previously found in sweat gland, airway and intestinal cells from cystic fibrosis patients⁴. The simplest explanation of the single-channel studies was that the cystic fibrosis gene product is a kinase-

activated Cl^- channel. But when the cystic fibrosis gene was eventually cloned and sequenced, it was found that the predicted primary structure of its product, CFTR (for cystic fibrosis transmembrane conductance regulator), did not resemble known ion channels already known. Rather, CFTR shared homology with a group of diverse ATP-driven solute pumps, fuelling speculation that CFTR might actively transport a regulatory molecule rather than serving as a Cl^- channel⁵. This broader view was able to explain alterations in other epithelial cell functions that are associated with cystic fibrosis, such as enhanced airway absorption of sodium ions and changes in mucin chemistry. Indeed, the fact that CFTR did not look like an ion channel even raised doubts about the primacy of the Cl^- channel defect in cystic fibrosis. ►

HIGH-TEMPERATURE SUPERCONDUCTORS

Whorls upon whorls

Laura Garwin

THIN films of the high-temperature superconductor $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ (YBCO) are packed with spiral structures measuring only a few hundred nanometres across. This observation, reported by C. Gerber and colleagues on page 279 of this issue, and independently by M. Hawley *et al.* in this week's *Science* (251, 1587; 1991), sheds new light on how these films grow, and may also reflect on their ability to carry high electric currents.

Superconducting films, grown epitaxially on a crystalline substrate such as SrTiO_3 , are the raw material for superconducting electronic devices. Film deposition is an energetic affair, in which atoms of the material to be deposited are evaporated or sputtered from a target, and encouraged to land on the substrate. Despite the violence of this process, the films so obtained are highly oriented and have smooth surfaces — at least as seen by the electron microscope.

Gerber *et al.* used the scanning tunnelling microscope (STM) to take a closer look at sputtered films of YBCO, and were rewarded with images like the ones shown here and on page 279. Each spiral staircase reveals the presence of a screw dislocation — a line defect in the crystal lattice that creates a step on the surface of the growing film. The dislocations themselves run down the centre of each spiral, and might be invisible even to the STM, were it not for the fact that they have acted as sites of preferential nucleation for the growing film: each spiral grows by the addition of atoms to the edge of the step.

Beyond their mere existence, the number of screw dislocations observed (over 10^9 per square centimetre) is also of interest. It is paradoxical that, for applications, there is nothing worse than a perfect superconductor. Just about all uses for superconductors involve the presence of a magnetic field, which enters the superconductor in the form

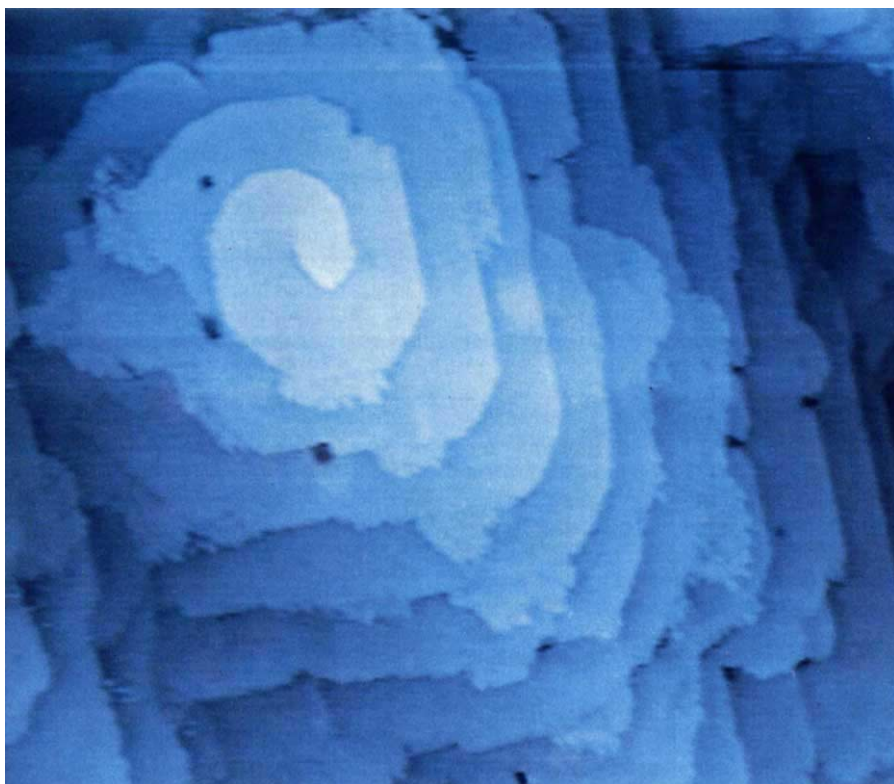
of quantized vortices, or 'lines', of magnetic flux. A flowing current exerts an electromagnetic force on these flux lines — a force that dissipates energy if the lines are free to move through the crystal lattice. Only when the flux lines are pinned in place, at grain boundaries or crystal defects, for example, can the superconductor carry a high current.

Gerber *et al.* show that, in low magnetic fields, the screw dislocations pin flux lines

sufficiently strongly to account for the observed high currents. In higher fields, the number of flux lines per unit area exceeds the number of dislocations, so that some other structure must provide additional pinning. Hawley *et al.* suggest that defects at the boundaries between spiral 'islands' could each pin several flux lines.

Both groups anticipate the objection that the microstructure they have observed might be peculiar to their own films, by presenting arguments for its probable generality. Each will surely be happy to see independent confirmation in the week of publication. □

Laura Garwin is Physical Sciences Editor of Nature.



Growth spiral at the surface of a 130-nm-thick YBCO film. The black spots at the growth steps are holes over 2 nm deep, which have evidently impeded crystal growth.

Confluence of the functional and structural themes of the story occurred with the success of genetic complementation experiments^{6,7}. In airway and pancreatic cells from cystic fibrosis patients, transient or stable expression of the cystic fibrosis gene produced a cAMP-regulated Cl^- conductance where none had existed before. Although these findings connected the Cl^- channel regulatory defect with CFTR, it remained unknown whether CFTR was a Cl^- channel or whether it regulated Cl^- channels already present in the plasma membranes of the epithelial cells. The new nonepithelial expression studies do not resolve this issue either, but they do impose additional constraints on CFTR function. The vaccinia-virus expression system used by Anderson *et al.*¹ appropriates the cell's protein synthetic machinery, ruling out CFTR-induced expression of other proteins (such as epithelial-specific Cl^- channels or regulators). This implies that the only factor necessary for a cell to mount a cAMP-activated Cl^- conductance is CFTR. Kartner *et al.*² provide us with single-channel recordings corresponding to this Cl^- conductance. By implication, the recordings represent the single-channel records of CFTR or of the Cl^- channel with which CFTR consorts.

These results leave in their wake unanswered questions about several earlier findings. If CFTR is a Cl^- channel, then which one is it? Epithelial cells have a variety of Cl^- channels, with different biophysical properties. Recordings of Cl^- currents in whole-cell patch-clamp studies reveal at least three conductances that are evoked by secretory and volume-regulatory stimuli⁸. Cell-attached channel recordings from pancreatic and intestinal cells provide evidence of a cAMP-activated channel whose properties are consistent with the cAMP-activated Cl^- conductance^{9,10}. The channel has a linear current-voltage relationship and a single-channel conductance of 10 picosiemens (pS). These are the channel characteristics that Kartner *et al.*² detect in nonepithelial cells transduced with the complementary DNA for CFTR and attribute to CFTR.

Yet our concept of Cl^- channel dysfunction in cystic fibrosis is based on protein kinase-induced activation of a 30–50-pS Cl^- channel with an outwardly rectified current-voltage relationship, the 'outward rectifier'. The failure of protein kinases to activate the outward rectifier in membranes from cystic fibrosis cells is a consistent finding (reviewed in ref. 3). Will the real Cl^- channel please stand up? This is not a trivial problem, because most biophysicists would agree that an ion channel has certain unique features, including its single-channel conductance, ion selectivity and blocker sensitivity. The 10-pS Cl^- channel and the outward rectifier differ in all of these characteristics^{8,10}. In general, channel regulators do not alter these properties, but instead affect the number of active channels or the level of their activity. Can CFTR behave as both the

10-pS Cl^- channel and the outward rectifier? Probably not. Proof that CFTR is a Cl^- channel awaits a successful reconstitution experiment in which CFTR is incorporated into an artificial membrane system (planar lipid bilayer).

A second unresolved question with the $\text{CFTR} = \text{Cl}^-$ channel equation is its dose-response relationship. Efficient expression systems can generate considerably greater amounts of CFTR in transfected cells than those which exist in native epithelia, perhaps corrupting normal cellular functions in doing so. If nonepithelial cells expressed an amount of CFTR more representative of airway cells, would a cAMP-dependent Cl^- conductance be detected? And what of the cellular location of CFTR? It is the relationship between plasma-membrane CFTR and Cl^- conductance that is of interest, and this has not, so far, been adequately defined.

Finally, are alternatives to the idea of CFTR being a Cl^- channel still worthy of serious consideration? We feel that CFTR could function as a regulator, although the results obtained from expressing CFTR in nonepithelial cells now certainly limit the possibilities. Ostensibly, CFTR must be capable of subverting a garden-variety Cl^- channel, directing it, if necessary, to the plasma membrane and conferring on it the ability to be regulated by cAMP. A suitable model arises from the observation¹¹ that endosomal membranes show a Cl^- conductance that is stimulated by protein kinase A. Can expression of CFTR promote its appearance in the plasma membrane? There are undoubtedly other models, but the important point is that the phenotype that we associate with secretory epithelial cells, namely a cAMP-activated Cl^- conductance, can be conferred on other cells by CFTR.

The simplest explanation at present is $\text{CFTR} = \text{Cl}^-$ channel. Definitive proof awaits positive results from the reconstitution experiments. In the meantime, we remain as the two mathematicians in a Sidney Harris cartoon. One, having sketched a complex, multivariable equation across the blackboard, comes to the equals sign. He turns to his colleague and says, "This is the part that always makes me nervous". □

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Bespoke flight

AFTER a recent air journey enlivened by overbookings, air-traffic delays and missed connections, Daedalus began to grumble that airline timetables these days are mere works of fiction. He now plans to do away with them altogether.

His basic idea is very simple. To make a flight, you phone up the booking system and say where and when you'd like to fly. A computer scans the incoming requests. When a plane-load has built up around a specific route and time, it invites subscribing airlines to 'bid' for that flight.

The strategy of bidding would be pretty subtle, and would have to be fully computerized. Fuel, crewing, the cost of airport 'slots', and the availability nearby of an aircraft of about the right size, would all have to be taken into account. If an airline already had a planned flight arriving at the airport around the proposed departure-time, for example, it could put in quite a low bid. By selecting the lowest bids, the computer will automatically interleave the proposed flights into the most economic overall flight-plan. As passengers announce themselves or change their minds, it will update that plan.

For the passengers will be part of the auction. Whenever a flight has been tentatively defined, the computer will phone them all with news of its time, proposed cost, destination, and current likely alternatives. Some will confirm their intention. Others will switch flights, perhaps holding out for a flight to fit their own tight schedule regardless of cost, or accepting a very cheap offer on a nearly-full flight going roughly in the right direction. The voice of the market, what it wants and can afford, what it will sacrifice for cheapness or pay for convenience, will speak clearly and efficiently — much more so than through the present rigid timetables, fixed fares and discounts with silly restrictions. And the computer will respond, by re-optimizing the global flight plan and presenting it again. Ultimately, all the passengers will be accommodated with an absolute minimum of air-movements. Expensive aircraft will be ideally matched to well-expressed demand by cheap telecommunications and information technology.

Flexible-response routing will give airlines the same sort of advantage that just-in-time delivery gives to a factory. No longer will aircraft fly nearly empty for lack of demand. No longer will passengers find themselves overbooked, or forced into circuitous journeys by inflexible scheduling. On popular routes, demand will build up so fast that flights will be almost as frequent and predictable as a scheduled service, and much cheaper. Only the most awkward and least-travelled routes will show serious volatilities of time and cost, as they should.

David Jones

Screw dislocations in high- T_c films

SIR — Epitaxial films of high-transition-temperature (high- T_c) superconductors have impressive critical current densities of $\sim 6 \times 10^6 \text{ A cm}^{-2}$ at 77 K. This suggests the existence of strong pinning sites for magnetic flux lines¹, but the nature of the most important pinning sites is not yet known. Pinning has been observed at twin boundaries, at the film surface and even in the layered crystal structure of the high- T_c oxides², but pinning by point defects and by dislocations has also been proposed¹.

By imaging epitaxial films of the high- T_c superconductor $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ (YBCO) with the scanning tunnelling microscope, we have directly observed screw dislocations at densities of $\sim 10^9 \text{ cm}^{-2}$ in seven of nine samples studied. In each case, the concentration of screw dislocations exceeds that in the substrate by three to four orders of magnitude, suggesting that high dislocation densities are an intrinsic property of sputtered high- T_c films. The dislocation density observed is sufficient to account for much of the pinning force in the films.

Nine films of YBCO were grown by standard hollow-cathode magnetron sputtering³ on {100} SrTiO_3 substrates (see table legend for details). X-ray diffraction showed the films to be c -axis oriented. Two pieces of each substrate were used for scanning tunnelling microscopy (STM), and in the case of films thicker than 100 nm, one was patterned by photolithography and wet etching with 2% H_3PO_4 into bridges ($8 \times 100 \mu\text{m}$) for

transport measurements. The bridges have T_c values ($R = 0$) of 89–90 K, resistivity ratios (300:100 K) of ~ 3 and critical current densities of $5\text{--}8 \times 10^6 \text{ A cm}^{-2}$ at 77 K and $5\text{--}6 \times 10^7 \text{ A cm}^{-2}$ at 4.2 K (1 μV criterion in self-field).

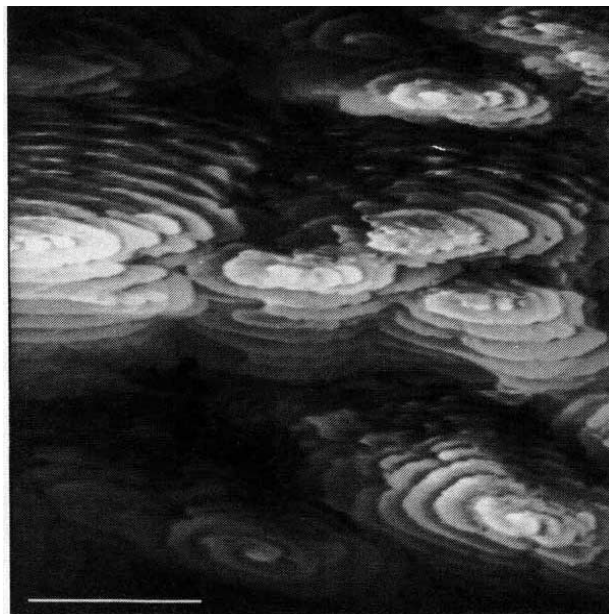
STM imaging was done without additional surface treatment at room temperature in air, using mechanically prepared $\text{Pt}_{80}\text{Ir}_{20}$ tips. Presumably owing to tip-sample contact, tunnelling with low gap resistances led to a deterioration of the tunnelling behaviour; for this reason, the gap resistance was kept at $> 3 \times 10^{10} \Omega$. The tunnelling current I_T was set to 20–30 pA at a tip bias voltage $|V_t| > 0.6 \text{ V}$. About one hundred images were taken at various locations on the samples. All data presented are unfiltered raw data.

The figure shows a typical STM image of a $750 \times 750 \text{ nm}$ surface area of a 120-nm-thick film (sample 3 in the table); 12 screw dislocations can be readily identified. Two films sputtered more slowly with a reduced discharge current (0.05 instead of

0.13 nm s^{-1}) have a somewhat lower dislocation density, and densities of $\sim 10^9 \text{ cm}^{-2}$ have been observed in films as thin as $\sim 12 \text{ nm}$ (sample 1 in table). Tunnelling on such thin films was found to be rather difficult, however, which is why no values are given for the dislocation densities of samples 8 and 9. In thin films, growth spirals developed fewer turns (≤ 1) than in thick films, in which up to 20 turns (sample 7) were imaged.

Most terraces around the dislocations in the figure are atomically flat and separated by growth steps one unit cell high ($\sim 1.2 \text{ nm}$). In other samples (such as no. 2), step heights of two or three unit cells dominate. The growth-rate dependence of the dislocation density, and the large difference in the dislocation density between the films and the

substrates suggest that the screw dislocations nucleate during growth at defects (for example, at stacking faults) or during island coalescence. Images like that on page 277 of this issue indicate that the screw dislocations serve as growth centres for the YBCO films: the presence of growth spirals emanating from the dislocation cores and the observed



STM image ($I_t = 10 \text{ pA}$, $V_t = 1.0 \text{ V}$) of an epitaxial, c -axis-oriented YBCO film (sample 3 in table) showing 12 screw dislocations of both signs. Scale bar, 200 nm.

interactions between growth spirals are consistent with crystal growth theory⁴.

The pinning force per unit length, f_p , on a flux line paralleling for a fraction α of its length a dislocation can be roughly estimated⁵ as

$$f_p(T) = \frac{\alpha\beta}{16\pi\mu_0} \frac{\Phi_0^2}{\lambda^2(T)\xi(T)}$$

where Φ_0 is the magnetic flux quantum, $\lambda(T)$ the magnetic penetration depth and $\xi(T)$ the coherence length. If the parameter β is set equal to 1, this equation describes the ideal case in which the order parameter of the superconductor drops to zero at the dislocation core and rises to its full value within a distance ξ . In practice, such ideal pinning conditions may not prevail, which is taken into account by employing $\beta \leq 1$. For c -axis-oriented YBCO films at 4.2 K in magnetic fields parallel to the c axis, the equation yields a pinning force $f_p \approx \alpha\beta \times 2 \times 10^{-3} \text{ N m}^{-1}$. In the critical state, f_p equals the Lorentz force f_L per unit length at the critical current density J_c : $f_L = J_c \Phi_0 = 10^{-3} \text{ N}^{-1} \text{ m}$ for $J_c = 5 \times 10^7 \text{ A cm}^{-2}$. This shows that with a reasonable $\alpha\beta$ product of $\frac{1}{2}$, the screw dislocations may account for the critical current density in low magnetic fields.

In agreement with the idea that for screw dislocation densities of $\sim 10^9 \text{ cm}^{-2}$, in low magnetic fields each flux line may be pinned by a dislocation, we observe no systematic dependence of J_c on the screw dislocation density in self-field. Above $\sim 0.02 \text{ T}$, however, the flux-line density exceeds the ob-

CHARACTERISTICS OF THE INVESTIGATED SAMPLES

Sample no.	Thickness (nm)	Growth rate (nm s ⁻¹)	Screw dislocation density* (10 ⁸ cm ⁻²)
1	12	0.13	27.5±3
2	60	0.13	14.5±2.5
3	120	0.13	13.5±2
4	130	0.05	9.5±1.5
5	150	0.18	10.5±1
6	140	0.11	9±1
7	150	0.05	8.5±1
8	12	0.13	—
9	12	0.13	—

Samples 1–4 and 5–9 were deposited from two different targets, on two different batches of SrTiO_3 substrates. All substrates were grown by the flame fusion method (Commercial Crystals, spec. atomically polished); their dislocation density is $3\text{--}5 \times 10^5 \text{ cm}^{-2}$ as measured by an etch-pit technique⁷. Each substrate was rinsed in acetone and propanol, and glued onto the heater of the sputtering chamber with silver paint. The sputtering parameters used were a substrate heater-block temperature of 750 °C, a total pressure ($\text{Ar}/\text{O}_2=2:1$) of 0.86 mbar, a plasma discharge of 150–170 V and 200–500 mA (450 mA corresponds to a growth rate of 0.13 nm s^{-1}) and aftergrowth cool-down in 0.5 bar O_2 lasting $\sim 1 \text{ hr}$.

*Average value at the film surface.

served dislocation densities, so that the behaviour of J_c in high magnetic fields ($J_c(2\text{ T}) \approx J_c(0\text{ T})/2$; $J_c(8\text{ T}) \approx J_c(0\text{ T})/4$) cannot be explained by the observed screw dislocations alone, but would instead be consistent with a total line-defect density (for example, edge dislocations) of $\sim 5 \times 10^{10}\text{ cm}^{-2}$, or an even higher point-defect concentration⁴. Preliminary results indicate that J_c starts to vary with magnetic field strength B (for $B \parallel c$) at fields of 0.1–2 T, depending on growth conditions.

Finally, we emphasize that our films are fabricated by a standard technique, suggesting that the observed surface structure is general to sputtered YBCO films. Consistent with this suggestion are transmission electron micrographs of sputtered YBCO/PrBa₂Cu₃O_{7- δ} multilayers⁶, which reveal an interface roughness comparable to the surface roughness observed in our STM studies.

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Beneficial effects of ghee

SIR — Milk fats contain small amounts of conjugated linoleic acid (CLA) isomers¹ which may have anti-carcinogenic properties². In India, ghee, the anhydrous milk-fats of cow and buffalo, has been traditionally considered as the most healthy source of edible fat. Ghee is manufactured by different

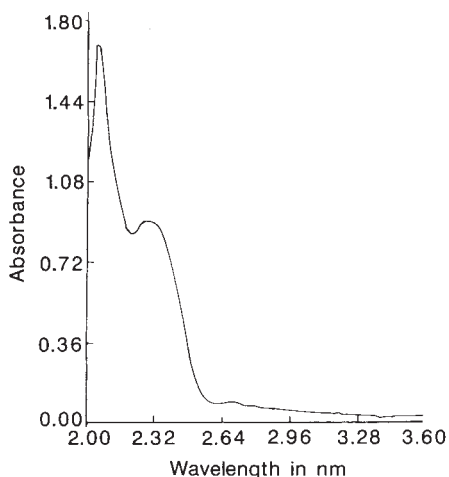


FIG. 1 Ultraviolet absorption spectra of ghee sample (*desi*) in iso-octane at 233 nm in 1-cm cell.

methods of clarification of butter or cream, in which milk protein is a normal constituent in the aqueous phase. Milk proteins provide hydrogen to the double bonds of linoleic acid during heating under anaerobic conditions and catalyse the formation of CLAs, similar to the microbial enzymatic reaction in the rumen³.

Milk fats extracted by a solvent mixture from cow or buffalo milk contained 0.6 and 0.5% CLAs, respectively, as determined by ultraviolet spectrophotometry method⁴. The extraction of fat from milk by a solvent mixture does not involve fermentation or heating, so the CLAs in these fats are due to the natural microbial enzymatic reaction in the rumen.

In the traditional (*desi*) method of making ghee, whole milk is converted into curd (*dahi*). We have shown that microbial fermentation during curd formation increases

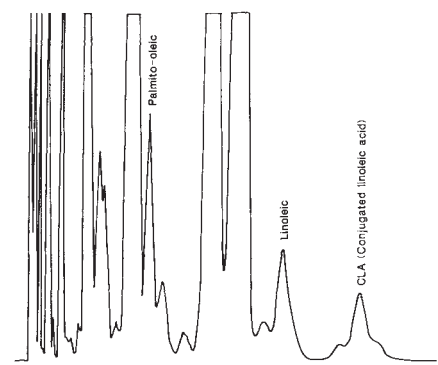


FIG. 2 Gas chromatogram of ghee (*desi*) sample. Identification of minor unsaturated CLA fatty acids.

the CLA content of milk fats to 1.0%. Heating of milk fats in the presence of proteinaceous materials, as in ghee making, is known to increase CLA content⁵. There is a further increase of CLA content (2.5–2.8%) in ghee samples when butter is clarified at higher temperatures (120 °C) than at the 110 °C (1.1–1.3%) traditionally used in villages to make ghee. In the creamery-butter method, the CLA levels were 0.9–1.8% in cow and 0.7–1.6% in buffalo ghee, respectively, at the clarifying temperatures of 110 and 120 °C. In the direct cream method, the levels were 0.6–0.7%.

We re-examined these findings in detail by liquid chromatography using dehydrated castor oil containing 9–11 octadecadienoic acid (c,c; c,t; t,t isomers) derived from ricinoleic acid as standard. In general, these CLA values (Fig. 2) were in very close agreement with those obtained by spectrophotometry.

Ghee is an important source of fat for most people in India; there is an annual produc-

tion of 800,000 tonnes, the bulk of which is by the traditional method. Because milk fat has a high content of saturated fatty acids, we would like to emphasize the beneficial effect of ghee as it contains high levels of CLA (2.5–2.8%).

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Hubble's calibration error

SIR — I should like to comment on the letter from R. N. Wilson in Correspondence (*Nature* **346**, 693; 1990).

Wilson is correct to advocate the use of the pentaprism test for ensuring that the primary and secondary mirrors of a telescope fit together and that the system has been corrected for spherical aberration. Test results with the mirrors of the 2.5-m Nordic Optical Telescope (NOT) prompted a small correction to the shape of the secondary, but we also used the test with the primary alone to ensure the correctness of its conic constant. Because the primaries of Ritchey-Chrétien telescopes are only slightly hyperbolic, deviating very little from parabolic shape, the pentaprism test can be applied without difficulty. The components required for our testing device cost about \$3,000.

Our primary mirror was finished so that 80 per cent of the light is concentrated within a circle 0.22 arc-seconds in diameter. It has turned out that the quality of the combination is also very good; despite the effects of atmospheric seeing, image diameters as small as 0.35 arc-seconds (FWHM) have been obtained.

As the person responsible for the optics of

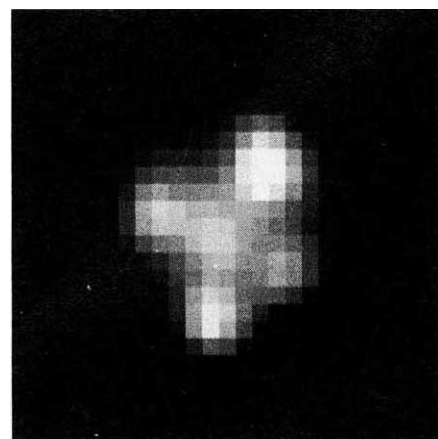


Image of the gravitational lens object Einstein cross, obtained with the CCD camera of NOT using red filter.

the NOT, I should also like to express my opinion of the way in which NASA and the European Space Agency (ESA) have advertised the imaging quality of the Hubble Space Telescope (HST), incidentally disparaging the performance of the NOT.

The 30 September 1990 issue of *Sky and*

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Telescope shows images of the same double star with HST and NOT which gives the impression that HST vastly outperforms NOT in image sharpness. Yet the HST image was processed so as to show only the core of the image, containing just 15 per cent of the light. In particular, the large faint halo, more than 2 arc-seconds in diameter, was not shown.

It is difficult to understand why a picture such as this should have been made public. The presence of spherical aberration must have been known when the false picture was made. Did those responsible believe that the faults of the HST mirrors could be concealed in that way?

Now that the truth about the HST is known, the widely distributed false pictures must give many people the impression that the imaging quality of the NOT is even worse. Clearly the NOT images shown were obtained during mediocre seeing conditions; they are by no means typical of those provided by the telescope. Indeed, the observing logbook shows that the seeing is better than 1 arc-second for 65 per cent of the time, and even less than 0.5 arc-seconds

for 7 per cent of the time.

It is especially ironic that NASA and ESA should have used NOT images to illustrate their claim of the superiority of HST over ground-based telescopes when NOT is one of the few telescopes that consistently give sharper images than HST in its present state. I believe that this unfair comparison has thrown mud at the reputation of NOT, which is why the astronomy and scientific community should know that the image sharpness of our telescope is second to none.

By way of demonstration, the object in the accompanying image of the gravitational lens object QSO 2237+0305, also known as the Einstein cross, has a maximum diameter of only 1.7 arc-seconds, of the same order as the single stellar images with NOT shown in the HST advertisement. With other telescopes, the four quasar images and the nucleus of the lensing galaxy are resolved only in exceptionally good conditions. For NOT, this is possible on most clear nights.

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Hydrothermal vents in Lake Baikal

SIR — Our discovery in mid-1990, in conjunction with a team from the National Geographical Society, of hydrothermal vents in Lake Baikal, confirms heat-flow and water-column temperature anomalies previously reported¹⁻³. The vents were found at a depth of 440 m on the sediment floor of Frolikha Bay (in the northeastern corner of Lake Baikal), at the foot of an east-west trending fault. Investigations were conducted from on board a ship fitted with two global positioning satellite receivers for navigation, with an accuracy of better than 100 m, 3.5 and 12

kHz echo sounders, a deep-towed camera and a CTD (conductivity, temperature, depth sensor).

Photographs from the area around the vents reveal that the centre of the vent field is covered by a near-continuous bacterial mat, consisting of long, thick white strands in a matrix of translucent whitish material. Temperatures of the sediment beneath the bacterial mat were greater than 16 °C, compared to an ambient temperature of 3.47 °C. The most obvious large organism in the region is a white sponge encrusting small cobbles at the periphery of the vent field. Coiled gastropods and whitish translucent amphipods are found among the sponges and on the sediment at the edge of the bacterial mat. Whether this 'vent' community consists of vent-specific taxa or is merely a dense concentration of background lake fauna is not known. However, no similar concentrations of organisms, or sponges with the same growth form, are seen in areas removed from the vent field (approximately 85% of the photographs). Patches of dark grey sediment and patches of sediment pock-marked by thousands of small holes (burrows?) are also seen at the edge of the vent field.

The fauna of Lake Baikal is unique in its high degree of endemism and in the number of species that have affinities to saltwater forms, suggesting that

the lake may have been connected to the oceans. How the taxa inhabiting the Lake Baikal hydrothermal vents fit into the biogeographical arena of vent communities needs more detailed investigation, but promises to provide interesting clues to the evolutionary history of these intriguing communities, as well as of Lake Baikal itself.

Unlike hydrothermal venting along the mid-ocean-ridge system, which occurs primarily along or near the axis of spreading, the hydrothermal vents in Lake Baikal occur along a flanking fault zone more than 18 km from the axis of the rift valley floor. This relationship between flanking faults and high heat flow is consistent with geothermal surveys in the central part of the tectonically similar East African Rift⁴. The difference in setting between oceanic vents and continental-rift vents is thought to be a response to the different quantities of magma beneath. Along, most mid-ocean ridges, where magma is plentiful and located primarily along the axis of spreading, axial fissures and faults act as central conduits for water entrances and exits (K. C. F. Aikman, D. Altman & J. Perlin, manuscript submitted). Under the continental rift, however, magma is scarce and the young rift is dominated by tectonic activity, for example, deeply incised, flanking faults. Magma bodies are either at great depth or nonexistent. Therefore meteoric water that enters flanking faults sinks to great depth, is heated by an enhanced geothermal gradient and then erupts back onto the surface along the same system of faults by which it entered the subterranean world. This fault-controlled hydrothermal exchange may have a substantial effect on the chemistry and the stability of the Lake Baikal water column. The tectonic setting of hydrothermal vents in Lake Baikal may provide clues to its degree of evolution as a continental rift.

Lake Baikal and its associated rift contain biological and geological evidence linking the lake's origin to the birth of an infant ocean. The degree to which Baikal has evolved from a purely continental arena into its transition between a lake and a sea can only be deciphered by additional exploration.

KATHLEEN CRANE

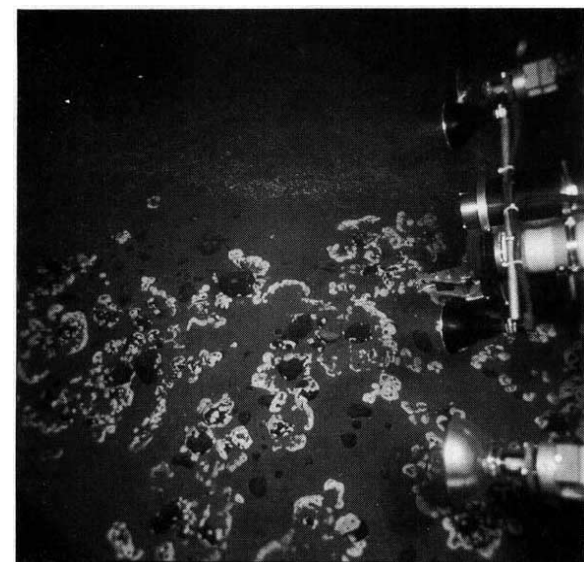
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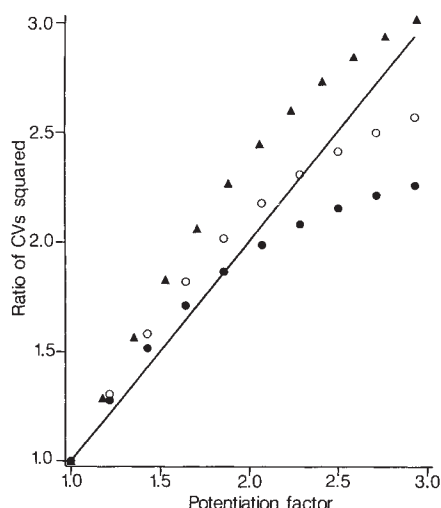


Low-angle oblique view of the Frolikha Bay vent site. In the foreground are sponges encrusting cobbles at the periphery of the vent field. In the background is a mat of filamentous bacteria resting on sediment closer to the centre of the vent field. Photograph taken by Emory Kristof, National Geographical Society.

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Is maintenance of LTP presynaptic?

SIR — Two recent suggestions^{1,2} that the synaptic enhancement underlying long-term potentiation (LTP) in neurons is maintained by presynaptic mechanisms are based on the fact that, given a binomial model of transmitter release at a single connection, changes in a quantitative measure of the variability of the postsynaptic responses (coefficient of variation) should be indicative of a presynaptic locus for the modifications. In both, the assumption was that in the preparation of hippocampal slices a single afferent was reliably stimulated by 'minimal extracellular stimulation', so that all failures occurred at the release step. In this case, one presynaptic neuron projecting to the target cell must be stimulated consistently while no other inputs are activated, even intermittently. Although



Relation between changes in synaptic efficacy (abscissa) and the reduction in coefficients of variation (CV-ordinates) after LTP, assuming an increase of quantum size alone. The presynaptic parameters used were, $n=9$, $p=0.6$ for ▲ and ○, and $n=7$, $p=0.6$ for ●, while the probability that individual cells were excited by the stimulus ranged, in each series, from 100 to 10%. Solid line, data interpreted as representing a presynaptic modification². ▲, $N=6$, $np=5.4$; ○, $N=3$, $np=5.4$; ●, $N=3$, $np=4.2$.

it is understood that the coefficient of variation method is a powerful but indirect tool (see refs 3,4), it seems reasonable to explore the alternative that the extracellular stimulus might excite several afferents, with there being intermittent failures of impulse initiation in some of them.

If the analysis developed by Bekkers and Stevens² for multiple inputs is expanded in this way, one can find similar results (potentiation equalling the ratio of the coefficients of variation squared) when quantal size, q , is the only parameter modified. This occurs if q at individual connections is enhanced as a nonlinear function of the fraction of time each afferent cell is activated, a formulation consistent with a hebbian model for potentiation. This is illustrated in the figure for

which it was assumed that several (N) cells were involved, all with the same initial values of q , n and p , the last two being the number of release sites³ and release probability, respectively. The predictions are similar to the experimental observations^{1,2}, with the agreement covering a broader range if the neurons have relatively large np products or more cells are involved. Also, assuming multiple afferents, one finds a wide range of parameter sets for which Poisson fits of evoked histograms appear, as in slice experiments², superior to simple binomials. In these situations, failures at individual connections would be hidden.

Thus, in the case of extracellular stimulation, the coefficient of variation does not reliably distinguish between pre- and postsynaptic sites of modification. The notion that maintenance of LTP has a major presynaptic locus even though the process is initiated postsynaptically is inherently attractive, as it emphasizes the unity of a synapse. It is to be hoped that this issue can be resolved by a more direct structural and quantal analysis of LTP at connected cell pairs, such as the preliminary data referred to in ref. 1, although this approach has met with limited success in the central nervous system, mainly because of the difficulty in resolving quanta^{3,4}. (A fuller version of our arguments will be submitted for publication elsewhere.)

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MALINOW AND TSJEN REPLY — Korn *et al.* use a detailed model to point out a limitation in analysis of hippocampal synaptic transmission^{1,2} based on the coefficient of variation (CV)⁵. This assumption, already stated explicitly in our paper¹, is that all synapses are homogeneous with respect to postsynaptic responsivity during a given epoch. CV analysis is analogous in this respect to noise analysis of ion-channel properties: both approaches give an over-view of statistical properties, but they require assumptions for quantitative estimates of unitary parameters. Channel noise analysis is often followed up by single channel recordings. Similarly, CV analysis can be complemented by other means for localizing changes in synaptic efficacy: (1) analysis of failures of transmission^{1,2,6,7}, (2) measurement of quantal spacing⁸, (3) analysis of the amplitude and

frequency of miniature excitatory postsynaptic currents^{9,10}, (4) assays of postsynaptic responsiveness by local application of transmitter^{10–12}. Clearly, several different approaches are desirable when studying LTP.

These additional approaches are not addressed by Korn *et al.* The analysis of synaptic failures is one relevant case. Their model predicts no change in failures, clearly contradicting published observations in conventional hippocampal slices with either minimal extracellular stimulation^{1,2,7} or intracellular stimulation^{1,6}, or in cultured hippocampal neurons. In each of these systems, the proportion of failures decreases sharply during LTP.

The model proposed by Korn *et al.* requires multiple afferent pathways with varying degrees of excitation failure. These complications were addressed in cell-pair experiments in hippocampal slices; a single presynaptic cell was reliably stimulated intracellularly during whole-cell recording from a post-synaptic cell (refs 1, 6, 7 and 13). In each of four pairs of synaptically connected cells, we saw increases in CV^{-2} , a decrease in failures and a shift in the amplitude histogram from a left-skewed form to a more symmetrical distribution (ref. 1, 6, 7, 13). These results, similar to those obtained with extracellular stimulation, provide evidence of presynaptic modifications during LTP, without ruling out possible additional postsynaptic changes.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. □

Technological *glasnost*

Zhores A. Medvedev

Science Policy in the Soviet Union. By Stephen Fortescue. *Routledge: 1990.* Pp.230. £35.

Science and the Soviet Social Order. Edited by Loren R. Graham. *Harvard University Press: 1990.* Pp.443. \$35.00, £27.95.

THE Soviet Union, as is made clear in *Science Policy in the Soviet Union* and *Science and the Soviet Social Order*, has half the scientists and engineers in the world, a fact which has been a source of pride for Soviet leaders. "But why" asks Harley Balzer in the second of these volumes, "with all that skilled personnel, does the Soviet economic system perform so poorly? Is the problem with the system or the people?" In the 1920s, 1930s and 1940s it was apparently the system that was mainly responsible. Now that engineering or academic qualifications are essential for recruitment and promotion of members of political elite and top management of the Soviet Union and in all its republics the system cannot be so clearly separated from the people. For decades the system used to suppress and punish independent individual initiative and to create the most restrictive environment for technical research and artistic creativity while simultaneously improving opportunities for advanced education. This led to an overproduction of intellectuals who in general do not occupy the same middle-class position as in less ideologically motivated and more technocratic western societies.

In *Science Policy in the Soviet Union*, Stephen Fortescue reviews the general structure of Soviet scientific establishment up to 1989. The book contains many useful tables and data on the numbers of scientists who work in the Soviet Academy of Sciences and its regional branches, in union-republic academies, in the universities and other institutes of higher education, and in applied ministerial research networks. In addition, there is copious information about vertical interactions and subordination in science, from the level of Politburo, ministers, presidiums, directors, academic councils, heads of laboratories or chairmen of the departments. It gives a comprehensive picture of how financial funds move through the numerous links from the budget to the individuals and details how scientists receive their academic degrees and other credentials. But it does not attempt to discover why this enormous and well organized hierarchical pyramid made of several million people produces so little high-quality research and so few technological breakthroughs.

Science and the Soviet Social Order is a product of many discussions sponsored by the Russian Research Center at Harvard University. It provides a very fresh and innovative analysis of the interaction between science and the political system. It tries not only to explain the peculiarities of the Soviet

system which wanted to create its own separate Soviet science based on principles of dialectic materialism and marxist philosophy, but also to explain the failure to achieve this. The view emerges that, by putting the political leadership in charge of science and technology, the Soviet system was sometimes very effective in concentrating efforts for high-priority projects like nuclear projects and space exploration. (Even here, the names of the leading scientists and engineers

Seat of Soviet science — Academy of Sciences in Moscow during the 1940s.

were kept secret and most of the credit for success was taken by the party leadership.) At the same time it also stimulated numerous pseudoscientific trends, for example, in human genetics as described by Mark Adams in his excellent essay on the Soviet nature-nurture debates. A very new and highly convincing perspective in explaining the backwardness of the Soviet R&D system is given in the first part of Loren Graham's collection in two well written essays saturated with ideas; by Frederick Starr (new communications technologies and civil society) and by Seymour Goodman (information technologies and the citizen). The authors have produced a very convincing and brilliantly presented analysis arguing "that autocratic and authoritarian regimes one-sidedly develop vertical communication links, while democratic societies require elaborate horizontal networks as well as vertical ones". They also show that the tsarist regime which monopolized the printing facilities and introduced censorship and state control for all forms of communication started the

same trend which later the communist regime developed to perfection.

In the postwar Soviet Union from Stalin to Gorbachev, successive governments developed a very elaborate legislation to prevent or to control all forms of direct horizontal communication between individuals, groups, institutions, factories and administrative units. The central governmental and political bureaucracy always had to be involved even if this meant the continuing growth of all the structures of central apparatus. This compulsory use of vertical communications even for trivial matters inevitably delayed solutions, reduced diversity and put the Soviet Union well behind the west in all forms of communication and economic development. It was not a political revolt which finally eroded the power of the party bureaucracy, but the information technology which governments were not able to control. Short-wave radios, tapes, video, copiers, fax machines and computers created the technological *glasnost* which made information a real power. In every single case the Soviet government tried to control the horizontal lines of communication, at first by promoting loudspeakers instead of radios, then producing limited-wavelength radios, making Soviet-made videos incompatible with western models, by the introduction of a single gateway for all computerized data entering the Soviet Union and by making the use of modems forbidden for individual owners of personal computers and by banning the sale of copiers. The main task of the KGB during the first five years of *perestroika* became the restriction and control of the proliferation of communication technologies. But the KGB was losing the battle.

In 1989 when these papers were written, the authors predicted the inevitable failure of systems in which vertical lines of communication are dominant, but they did not know how long it would take. In fact, this failure happened much sooner than they ever expected. It was swift and dramatic in eastern Europe, but complex and painful in the Soviet Union. The abolition of censorship, the legalization of copiers and computer printers for individual use and the democratization of local elections happened nearly at once during late spring and summer of 1990 and suddenly reduced the power of central government. Without capability to control the existing developed horizontal links in science, economy and even trade, the imperial centralized structure started to fall apart. The government lost its ability to shape society, as predicted by F. Starr. But his expectation that the society instead will start to shape the government is yet to be fulfilled. Soviet society, so complex in its ethnic, cultural and political structure, is now moving more towards anarchy than to a new better and more advanced social order. □

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Super plants

John R. S. Fincham

Risk Assessment in Genetic Engineering: Environmental Release of Organisms. Edited by M. Levin and H. S. Strauss. McGraw-Hill: 1991. Pp. 403. \$39.95, £37.95.

AT the heart of the debate about the hazards of genetic manipulation is a fundamental question about evolution, namely the extent to which the potentialities of organisms are restricted by their own genetic systems. To put the question in teleological terms, are organisms divided into mutually isolated species for their own good, to avoid disruption of their selected blends of mutually adapted genes, or are interspecific barriers restraining the emergence of new and highly competitive life forms? Or are both propositions true, the first as the general case and the second as the rare but important exception?

Risk Assessment in Genetic Engineering hardly addresses these basic issues, but it does give a good picture of the state of the risk-assessment industry in the United States, where environmentalists, government agencies, firms of private consultants, lawyers and academics, not to mention the companies attempting to test and market their products, are locked in expensive (or lucrative) controversy and negotiation. Levin and Strauss's compendium consists of 17 chapters by different authors, writing from a variety of different standpoints — scientific, technological, administrative and social/political. The subject matter is confined to plants and the control of plant pests, presumably because it is only in this area that environmental release of manipulated organisms is in prospect.

The earlier chapters review the conceivable hazards from different applications of genetic engineering — engineered organisms and toxins for pest control, safety of transgenic plants as food, use of viruses to mitigate viral infections, horizontal gene transfer, the cultivation in open fields of transgenic plants. Several of the authors make recommendations on precautions, either during preliminary tests to assess hazards or after full-scale release.

Different authors manifest different levels of excitement. Kathleen Keeler and Charles Turner, in their long chapter on 'Management of Transgenic Plants', foresee both great benefits and great dangers. "We may expect to find transgenic plants growing as crops, in forests, in pastureland, in urban settings as ornamentals, on roadsides to combat erosion"; the transgenes will "run the gamut", they say. At the same time they see "immense potential for problems from human dispersal of transgenic plants in the next century". The most serious are to do with transfer of invigorating genes to weeds,

but the authors do not rule out the possibility that the engineered crops themselves may have increased invasiveness and recommend careful monitoring of the countryside adjacent to the new crops and the preparation in advance of measures for wiping out naturalized colonies. They are also worried that desirable wild species will lose biological diversity as the result of invasion by highly selectable transgenes. One cannot help thinking that the expense of dealing with this degree of worry is likely to price most transgenic plants out of the market.

James Fuxa, in his chapter on the use of engineered organisms (viruses, bacteria, fungi) for biological control of insects, also identifies a range of hypothetical risks, but seems somewhat more relaxed about them. "How many data are sufficient before an environmental release?", he asks, adding, with admirable frankness, "There is no good answer". He thinks, nevertheless, that limited release without disaster of organisms designed for low persistence in the environment will build confidence to the point where selective pathogens intended for persistence will become acceptable. "Zero risk will not be possible", he says, "nor should this impossibility prevent releases."

The trouble is that the concerned layman, not to mention the militant environmentalist, may be disinclined to accept that genetic engineering is worth any risk at all. Robert Wachbroit ('Explaining Risk') emphasizes the difficulty that laymen often have in understanding probability estimates; indeed, he seems not to understand them very well himself. A later chapter, by Joseph Fiksel, deals with the use of computerized knowledge systems in risk assessment. Useful as these may be for calling to mind relevant considerations, their usefulness must depend on the quality of the expert knowledge fed into them.

The problem of reaching agreement on what is relevant and realistic is well illustrated by Jonathan Naimon's chapter on the role of 'expert panels' with special reference to the furore in California concerning 'ice-minus' *Pseudomonas*, designed to protect fruit crops against frost damage. This product of genetic engineering has no transgene at all but only a controlled deletion of the gene coding for an ice-nucleation protein. It must have been quite a challenge to those trying to identify risks. One of the experts rose to the occasion by suggesting that wild-type 'ice-plus' bacteria in the atmosphere might be catalysts of rain, and that their replacement by ice-minus cells on the leaves of fruit trees could jeopardize the Central Californian rainfall. Presumably the next challenge is to devise a test of that hypothesis.

"One can only hope that reasonable attitudes will prevail", writes Fuxa. Everyone can say "Amen" to that. □

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Dated effort?

Maurice E. Tucker

Principles of Stratigraphic Analysis. By Harvey Blatt, William B. N. Berry and Scott Brande. Blackwell: 1991. Pp. 512. £39.50, \$49.95.

STRATIGRAPHY has long been regarded as one of the less interesting, very traditional topics of an Earth science degree, lots of formation names and fossil zones to learn with little obvious significance. To call a geologist a stratigrapher was once something of an insult, a person with little specialization who mapped a bit and stamp-collected rocks and fossils but had no real feel for the palaeontology, sedimentology or geochemistry of the rocks he/she was studying. But that has changed in the last decade or so, with the subject now very diverse and technical, requiring a jack-of-all-trades or a team of people to tackle stratigraphic problems. Stratigraphy has also had a great boost in the last few years with the development of sequence stratigraphy, a new way of looking at the larger-scale geometries of sedimentary rock packages. Sequence stratigraphy is an approach used extensively by the oil companies to predict the distribution of potential reservoirs and source rocks. Against this background then, any new textbook on stratigraphy will be looked at with great interest by college lecturers and students alike, as well as professional geologists.

Principles of Stratigraphic Analysis by American university professors Harvey Blatt, William Berry and Scott Brande briefly reviews the techniques used (field-work, fossils, seismic, wireline logs), describes sedimentary rocks and their depositional environments, outlines the tectonic controls and basin formation, and discusses economic resources (oil, coal, salt, sand). Chapters are also provided on geological time and correlation, distribution of organisms, evolution and biostratigraphy. The book does bring together a lot of useful information, enabling the student easily to get a feel for the breadth of the subject. There are several more comprehensive texts available on sedimentary rocks and depositional environments, at less than half the price, but the sections on the palaeo/biostratigraphy side do include material not readily accessible. But in spite of this, the book does have a lot of drawbacks.

Surprisingly, there is no presentation of the concepts of sequence stratigraphy even though these important ideas have been at the forefront of geological research for at least five years. The omission drastically reduces the value of the book to students. There is no excuse for the lack of sequence stratigraphy. Although the main book on the subject was published in 1988 (*Soc. Econ. Paleont. Miner. Spec. Publ.* 42) and ideas are still developing, there has been ample

time to include all the principles in this book, which has a 1991 publication date. In fact, a general feature of the book is that most of the cited references are pre-1987; there are only a few 1988. This is curious; it should not take three years to publish a book. Indeed, in addition to the content, the book has a very dated, early 1980s look about it. The old-fashioned single-column layout used results in much wasted space — a two-column format would have been more attractive and would have given flexibility for figures, photos, captions and tables. Similarly, it is a shame that the authors did not spend more effort to get their own photographs and devise new figures rather than borrowing from other people's work. Many of the figures that are used are specific examples of a feature containing too much local information and lacking explanatory text. The inclusion of an

author index in a book of this type is worthless, except perhaps to academics looking for citations of their own work. Another irritating feature of the book is that each chapter has its own reference list; students much prefer a single bibliography which wastes less space and is easier to use.

Principles of Stratigraphic Analysis does contain some useful material but an opportunity has been lost to make it the stratigraphy text for the 1990s. It is also far too expensive for British undergraduates to buy, and presumably North American students too. There should have been more consultation during the preparation of the book and stronger editorial control. The jacket design is brilliant. □

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A cause for concern

R.W. Battarbee

Acid Rain and Acid Waters. By Gwyneth Howells. Ellis Horwood/Prentice Hall: 1990. Pp.214. £39.95, \$79.95.

In *Acid Rain and Acid Waters* Gwyneth Howells refers to acid rain as a *cause célèbre* and one that since 1972 after two decades of intense research has "generated much heat, but not a great deal of light". Most scientists involved in the acid-rain debate would disagree but I know what she means.

Until recently Dr Howells was head of biology at the UK Central Electricity Generating Board's research laboratories directing a comprehensive research programme on acid deposition and its effects. She became a leading and much respected scientist on the acid-rain circuit, representing and effectively defending the interests of the power generating industry. As a consequence she has acquired a substantial knowledge of the disparate specialist sciences involved in this complex issue much of which is distilled in this book.

The book aims to give a scientific review of acid rain and its effects, using examples from both Europe and North America. It covers emissions, transformations, deposition of acidity, and the effects of acid deposition on vegetation, soils, water quality and aquatic biology, and includes a chapter on reversibility. Each chapter presents an extensively referenced literature review and finishes with a list of unresolved issues and a summary of principal findings.

Much of the material is of a factual nature, and, except for the curious decision to ignore some of the excellent work carried out in Britain, it is presented in a relatively straightforward way. At the heart of the book is the debate which has caused passions to run high over the last 15 years or so: the extent to which problems of surface-water acidification are due to acid rain. The general public, and indeed many scientists, may not be aware of the vigour with which alternative hypotheses, chiefly concerning the roles of natural acidification processes and land-use changes, have been put forward. These alternative explanations have been understandably espoused and promoted by power

Trees stripped bare — a forest in Czechoslovakia showing extensive damage as a result of acid rain.

generating utilities both in Britain and the United States.

It is in this politically important area that the book is most seriously flawed. Despite protestations that she is taking an "objective stance" based on the "scientific peer-reviewed literature" the author has clear sympathies, and uses the literature selectively to foster those sympathies. For example, in an

attempt to minimize the extent of acidified waters in Britain, data collated by the UK Acid Waters Review Group are presented for the measured pH change of rivers between 1970 and 1984. The author states that "a searching analysis of 75 data sets for monitored river sites in susceptible areas has shown that a trend of increasing acidity for the 10 years prior to 1986 could be found in only six". But it is not pointed out, as members of that Review Group acknowledge, that all the monitoring sites were in the nonsensitive lowland reaches of the rivers (some had pH > 8), and that in any event no decrease should be expected because acid emissions in the Britain were falling during that period.

Nevertheless, despite these biases there is a clearly stated general acceptance of the damage caused by acid deposition to sensitive ecosystems, and the penultimate chapter of the book is consequently devoted to a consideration of reversibility and recovery strategies. Here the author is of the opinion that emission control "will have limited benefit, especially within the next century" because of long-term depletion of base status and the historical accumulation of sulphur in soils. Strangely, the field evidence she cites in the chapter does not support this conclusion which suggests that lakes can respond rapidly to a reduction in acid deposition. Instead she prefers the evidence for a slow response derived from the forecasts of process-based models. This preference allows the author to argue, with some zeal, for liming as an alternative restoration

strategy. Unfortunately the disadvantages of liming, such as the additional ecological damage liming can cause and the need to repeat liming frequently, are not discussed.

In her conclusion then the author is in favour of only a modest amount of emission reduction, and, in her promotion of liming and catchment management, is dismissive of the ecological need to protect biotic diversity and restore natural communities. Moreover, in focusing only on a solution to the surface-water problem her conclusions myopically fail to embrace the wider environmental benefits of reducing acid emissions in particular and energy generated from fossil fuels in general.

Acid Rain and Acid Waters is a disappointing book, strong on facts and figures, but not really reaching the heights of scientific clarity and objectivity aspired to in the opening exchanges. □

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How to teach chemistry

John Coyle

Essentials of Molecular Photochemistry.

By Andrew Gilbert and Jim Baggott. Blackwell: 1991. Pp.538. £17.95, \$44.95.

THE title *Essentials of Molecular Photochemistry* suggests an intentional comparison with Turro's classic text *Modern Molecular Photochemistry*, and certainly there is a similar balance in the two books between organic photochemistry and the physical and theoretical ideas on which to base a good understanding of the reactions. Andrew Gilbert and Jim Baggott have succeeded in producing a text that may well be a standard for this decade. Those academic institutions which offer a substantial undergraduate course in photochemistry should find this book a strong contender for a course text, and where photochemistry is a small component of the course, the book should be on the reading list. There is so much that reinforces other aspects of chemistry, such as models of molecular electronic structure, quantum theory, organic reaction mechanisms and kinetics, and my view is confirmed that photochemistry can be used as an effective teaching vehicle for a substantial portion of what undergraduates are expected to learn about chemistry.

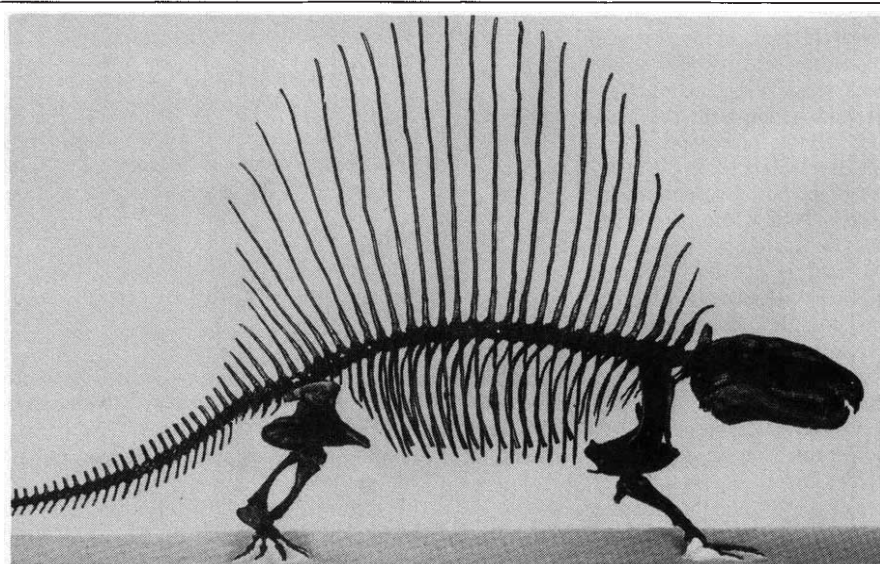
In the second part of the book, the descriptive organic photochemistry is organized according to functional group types — unsaturated carbon systems, carbonyl compounds, aromatic compounds, nitrogen-based systems, other classes, and photo-oxidation as a separate chapter. This is an approach that I have consistently advo-

cated, because it ties in well with the way organic thermal reactions are generally presented, and it makes sense in terms of the theoretical grounding required for each group.

If I do have a general criticism, it is one I would level also at a wide range of organic chemistry texts, not only photochemistry texts. An appreciation and understanding of the chemistry requires some familiarity with one or more theoretical models for describing electronic structure and changes in electronic structure. If the book is read sequentially from the beginning, however, the scientist with an interest in practical science may well lose heart before he gets to the 'real' chemistry after about 200 pages that include the Schrödinger equation, vanishing integrals, perturbation theory, equations for integrated absorption coefficients, Q-switching, Marcus theory, and much more. The premise seems to be that science theory needs to be taught before the practice of science, and my contention would be otherwise. What I would do to redress this somewhat in the current instance, is to whet the reader's appetite with an introductory overview of some important or unusual reactions that can be accomplished photochemically, without theory or explanation at this point. Then the reader might be better motivated to work through the 200 pages deemed necessary to help him appreciate the chemistry better.

I do not expect to change chemistry teaching habits overnight, so my earlier conclusion stands — this is a worthwhile successor to Turro and will serve the organic photochemical community well for several years to come. □

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A complete skeleton of a dimetrodon found in Texas and dated to the Late Permian (about 245 million years before present). One of 1,300 colour photographs to be found in *The Illustrated Encyclopedia of Fossils* by Giovanni Pinna and published by Facts on File, priced at £21.95, \$35.

Exploring the still unexplored

Antarctica remains a largely unknown continent. There should be better coordinated research on globally important problems — and the discontents occasioned by the Antarctic Treaty should be defused.

It is a familiar truism that the Antarctic is the last remaining part of the Earth's solid surface above sea level that remains to be explored. The Antarctic is also, from time to time, described as the last great natural laboratory on the surface of the Earth and as a natural wilderness with unique characteristics. But although eighty years have passed since Amundsen (from Norway) and Scott (from Britain) made their way with difficulty to the South Pole, there is a sense in which not much has changed in that remote region.

Of course, the number of bases at which research is undertaken has grown considerably, and it is now commonplace that handfuls of people overwinter on the ice. More general interest in the region has grown enormously, partly because of its potential as a source of materials (from fish to minerals) of possible value and, more particularly, because of governments' inclination to the belief that if the Antarctic Treaty constitutes a kind of club conferring privileges on its members, they had better also qualify for membership by programmes of Antarctic research, however modest.

The pages that follow are first-hand accounts, by regular contributors to *Nature*, of visits to Antarctica. Of necessity, no single Antarctic programme is comprehensively described, while several important national programmes are not even mentioned, or are mentioned only in passing.

What these accounts do provide is a sense of the flavour of Antarctic research. The old-timers do not fully welcome the way in which facilities (and creature comforts) are being improved with the passage of time.

Change, of course, is well known to be unsettling even to those ready to acknowledge that change is unavoidable. Yet in the Antarctic, there are several pressures in the direction of change, among which the most severely practical is the remoteness of Antarctica. The construction of landing strips is an obvious response.

French plans to that end seem to have attracted particular criticism, mostly on the grounds that landing-strips must damage the environment. But that conclusion is not

environment. But that conclusion is not necessarily correct. There are the strongest possible reasons for the careful limitation of the damage done to the natural environment of Antarctica, not the least of which is the notorious fragility of the landscape and its components. But there are also good reasons why, if Antarctica is a natural laboratory, it should be exploited in that role vigorously and systematically.

There is much to do. Part of the trouble is that the investigations most easily undertaken are those which can be sited at accessible places. Much more is known of the Antarctic Peninsula than of the continent proper. And there are difficulties about the collection of data about all but the most rudimentary physical variables that can be welded into comprehensive continental time-series. Observation points are geographically dispersed, the maintenance of equipment from one year to the next is not a simple matter and, in a research environment in which there is a natural temptation for national programmes to emphasize the novel in successive annual programmes, continuity is not naturally engendered.

This state of affairs is all the more disturbing because the importance of Antarctica in the global climatic balance is now more than ever recognized. Since the first suspicions that increased carbon dioxide in the atmosphere might provoke an increase of temperature, there have been three particular reasons why the continent has been singled out for special attention.

First, as the largest remaining glacial ice-sheet, Antarctica (with the smaller Greenland icecap) is the only plausible source of the water which, added to the oceans, might induce an unacceptable increase of sea level. Particular attention has been directed to the stability of the West Antarctic Ice Shelf, which is partly grounded offshore, and which might in some circumstances slump catastrophically, dumping a large quantity of ice into the Southern Ocean. Second, according to the climate modellers, the polar regions should be more sensitive to a global warming trend than are the mid-latitudes, implying that Antarctica should be a sensitive indicator of climatic change. Finally, the balance of ice accumulation in the Antarctic must be linked in important ways with processes of climatic change, but in ways that are not properly understood, and which are not accurately predicted: global warming should increase the rate of snow precipitation on the continent, but also increase the rate of ice removal at the edges, and the dif-

ference between two such large numbers cannot be accurately calculated when the relevant data are so sparse.

In circumstances such as these, logic would suggest that there should be an internationally coordinated programme of observations directed at the collection of data bearing in different ways on these inter-linked questions. Unfortunately, reality falls a long way short. The nearest approximation to a coordinating mechanism is provided by the Scientific Committee on Antarctic Research (SCAR), an offshoot of the International Council of Scientific Unions (ICSU) which is itself an offspring of the planning for the International Geophysical Year.

But SCAR has no research funds of its own to spend. Its budget of roughly \$250,000 a year, provided by contributions from its 28 member states, is almost entirely consumed by the cost of servicing a handful of expert committees concerned with its special programmes.

One particular consequence, for example, is that the movement of ice in West Antarctica is much less well known than its potential importance for global sea-level would suggest would be ideal. Part of the difficulty is that West Antarctica is inaccessible even by Antarctic standards, and that the area that would have to be covered by a meaningful study is in any case vast. (The Australian expedition is carrying out such a study in a much more accessible part of the ice-cap — see page 304.)

For reasons such as this, the prospects of early study of West Antarctica appear to centre on the use of remote sensing of the height of the ice surface by radioaltimetry from a dedicated Earth satellite. But as yet there are no plans for such a satellite, while it requires very little imagination to tell how many years would have to go by before it would be possible to infer from accurate contours of the ice surface how quickly the ice is flowing towards the coast.

This is not a criticism of SCAR as such, or of the informal bilateral and multilateral arrangements for collaboration in the Antarctic that seem to have been a conspicuous feature of the past few years. Rather it is that the members of SCAR (28, a subset of the 39 members of the Antarctic Treaty) have not yet committed themselves fully to the principle that the globally important problems in Antarctica would be more effectively tackled by careful advanced planning and coordinated execution.

When it is customary for people and governments of all kinds to acknowledge that

This survey of Research in Antarctica has been written by Christopher Anderson (United States), Peter Coles (France) and Tania Ewing (Australia), with contributions from temperate latitudes by Peter Aldhous (London) and Steven Dickman (Munich). *Nature* is grateful to the relevant national research organizations for their assistance in this projects as well as to the individuals named in the following pages.

Antarctica probably lies at the centre of many globally important problems, should not at least a fraction of the substantial sum of money now spent on Antarctic research be spent in such a way? Otherwise, the temptation to keep on making the easily made observations will be almost irresistible.

Much the same is true of the study of the ozone hole, the phenomenon by which there is a sudden decrease to virtually zero of ozone in the upper atmosphere at the onset of the Antarctic spring each year. This phenomenon, first recognized in 1986 by ground-based observations from the British Antarctic Survey's base at Halley Bay, appears to be confined geographically by the polar vortex, whose position changes with the progress of the seasons and which may vary from one year to another.

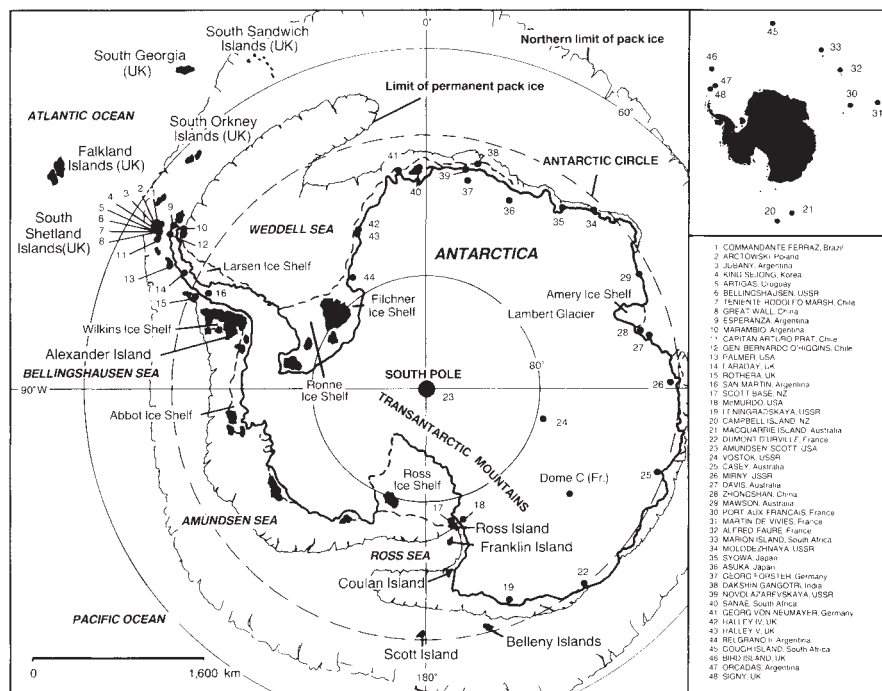
In an ideal world, a judiciously chosen network of observing stations would be the best way of pinning down the phenomenon, and of assessing more accurately than is possible at present the destructiveness of CFCs for natural ozone in the stratosphere. Will SCAR be given the muscle to prompt a coordinated study of that kind?

Informal collaborations seem, luckily, to have formed themselves into a more coherent pattern in the study of the Southern Ocean. Given that the Antarctic is both the chief source of the cold bottom water found in the deep oceans even at temperate latitudes, and that the Southern Ocean is also remarkably productive as far as various forms of planktonic life are concerned, this good fortune is much to be welcomed.

The following pages illustrate, for example, the attention being paid to krill, both by those who wish to study their behaviour and population dynamics and those who seek to fish them as a source of protein. But even after more than two decades of observation, too little is known of them to form the basis of a compelling conservation policy. By comparison, even less is known of the movement of water masses off the continental shelves of Antarctica into the deeper oceans to the north.

The plea that SCAR, or some such body, should be better equipped to formulate, perhaps even to mount, more tightly integrated programmes of research in Antarctica is not, of course, to deny the importance of the diversity of research projects that springs from the proposals of individual investigators across the world. So much should be plain, for example, from the way in which it has been recognized that the Antarctic is one of the most prolific sources of meteoritic material (pages 300 and 308), largely on the strength of Japanese discoveries a decade ago of physically substantial meteorites in the surface ice.

Research projects of an individual scale have an important part to play in the evolution of the Antarctic Treaty, the nearest thing there is to a law of the Antarctic. Much has changed since the treaty was signed by its twelve founding members in 1959. (The



Antarctica, points 1-48 showing the research stations active during winter 1990.

treaty entered into force two years later, after it had been ratified by its signatories.)

Even ten years ago, when the membership had grown to a score with the accession of India and Brazil, the treaty members cut the figures of members of an exclusive club, to which others might be admitted only after proof of a serious interest in research. Now, with the accession this year of Switzerland (which has managed to find a way of belonging to an international treaty although, not yet, to the United Nations), there are 39 members of the treaty, which no longer seems an exclusive club.

What seems to have happened is that the long-standing members of the treaty, and especially those (such as the United States) who regard territorial claims to designated parts of the Antarctic Continent as spurious but potentially dangerous, have recognized that the best way of avoiding squabbles over sovereignty in the future is to expand the membership of the treaty beyond the numbers of those with staked-out claims. It is as if a company seeks to dilute the claims of awkward shareholders by seeking extra equity to others.

Moreover, it is no longer necessary to establish a national research base in Antarctica to become a member of the treaty. It will suffice, on present policies, for a government to make a significant commitment to research and to make arrangements for researchers to carry out the work by using facilities, research vessels or land bases, maintained by other governments. Among other things, this principle has the benefit of reducing pressure on the land environment.

This, unfortunately, does not imply that all contentious issues have been buried. Last November's meeting in Chile, for example, publicly revealed the continuing disagree-

ment among the parties to the treaty about the arrangements there should be for exploring for minerals in Antarctica. After two years of painful negotiations, Australia and France defected (as their negotiating partners see it) to the view that Antarctica should be preserved as a perpetual wilderness, part of the international heritage.

One view of this negotiation now in abeyance is that, in the absence of an agreement of any kind on minerals exploitation, and with no reference to the preservation of the environment in the original treaty (except for the "preservation and conservation of living resources"), there is nothing at present to prevent people drilling holes in the ice and below in the search for economic deposits of minerals.

For the time being, fortunately, the difficulty (and cost) of mounting even scientific expeditions in the Antarctic is so great that there is probably time to carry through the negotiations to a satisfactory solution before the exploration ships set sail. The obvious compromise between the conservationists and the explorers would be a stop-gap convention requiring that all substantial construction works in Antarctica should be notified in advance to an appropriate authority with power to investigate the consequences and to forbid them if thought fit.

That is not the roseate view it may be thought. The plain truth is that the Antarctic is not an hospitable place. Those with research experience there are most acutely aware of the difficulties, while the framework of the treaty, while asserting that sometimes overlapping claims to sovereignty in Antarctica will not be extinguished by treaty membership, has in reality served to make compromise acceptable. With luck, it should continue to serve that purpose. □

Designing the next South Pole station

Washington

THE US South Pole station, a battered clump of buildings under an unheated aluminum geodesic dome, is operating on borrowed time. Snow and ice removal crews now spend almost the entire Antarctic summer unburying the station from the drifts of the previous winter. They are forced to bulldoze the snow half a mile away to avoid the worsening 'bowl effect' that has raised the surface around the station by some five metres in the 20 years since its construction. Each year, some 100 researchers and support personnel are squeezed into facilities originally designed for half that number.

Faced with the limitations of the current station, the National Science Foundation (NSF) is preparing to propose a major new facility at the South Pole. It has commissioned preliminary design studies from the Army Corps of Engineers' Cold Regions Research and Engineering Laboratory (CRREL) and Metcalf and Eddy, Inc., a private engineering company. NSF also asked the American Institute of Architects (AIA) to run a design contest for student proposals which drew 167 entries.

Late last year, NSF received the Metcalf and Eddy and CRREL reports, and when a winner was chosen, the top AIA proposals. NSF officials are now reviewing the designs in anticipation of selecting one approach and commissioning an engineering study. They hope to have settled on a single design by the middle of the decade.

The challenge of building a major new base on South Pole is considerable. It is the harshest climate on Earth, with temperatures that can fall to -100°F and ice storms that can bury small structures in days and leave huge snow drifts against larger buildings. The Sun rises once a year, on 21 September, and sets again six months later, leaving half the year in perpetual night.

Given that the polar environment rivals only outer space for inhospitality, it is not

surprising that many of the designs that have been proposed for the new base resemble space stations. (The winners of the AIA contest are from the University of Houston's Sasakawa International Center for Space Architecture.) In winter, when workers, even in full polar gear, can function in exposed air for only minutes at a time, stepping outside the station takes on the ritual of a space walk.

Although the submitted designs are all in the conceptual stage, the critical issues for a new South Pole station have already been identified, and several approaches have emerged to meet them.

Layout

The existing base is dominated by the four-storey dome, which has recently been renovated and is expected to remain a part of the next station (although all the proposals include elevating it by at least five to ten metres). Half-buried vaults, containing fuel, vehicles and the power plant, stretch in a line for some 250 metres beside the dome.

One of the three Metcalf and Eddy designs expands on the existing layout, replacing the row of vaults with two parallel rows of buildings on stilts, with the dome between them. Elevated and enclosed walkways would connect the buildings to the dome to complete the H-shaped layout.

Another Metcalf and Eddy proposal would build a second dome near the first, moving winter living and work facilities into the new structure and relegating the old dome to summer quarters and storage space. The company's third proposal would lift the dome atop a pentagon-shaped ring of two-storey raised structures, creating a tower some seven storeys tall.

CRREL has proposed a single line of raised, two-storey structures, linked by walkways to the dome in the middle. An internal column, required to raise the old dome, would serve double duty by providing

access to a small control tower or science station that would cap the structure.

Several of the student designs expand the dome concept to a raised geodesic sphere. (Engineers say that such a structure, while aesthetically appealing, would be prohibitively expensive to build at the Pole.) But the winning design, by John Major and Peter Dorsey of the University of Houston, is based on purely practical concerns. The students proposed a long, wing-shaped structure at ground level.

Facing the prevailing wind, the building's airfoil profile is designed to create a low-pressure zone at its leading edge to resist snow drifts.

Snow resistance

Snow drifting has turned out to be the most serious problem facing the current structure, and concern about snow accumulation dominates the proposed replacements. Wind-tunnel studies show that design decisions can make the difference between a structure that will be essentially buried by snow in a few years and one that can last for decades with little maintenance. With the exception of the student wing-shaped design, which depends on aerodynamics to stay clear, all the structures would be lined up parallel to the prevailing wind, which blows at a relatively constant 5–10 knots most of the year.

Elevated structures would usually have exposed support columns, to minimize drifting by encouraging a venturi effect in the area beneath the buildings. The existing dome, which has no built-in floor, would be raised on a man-made snow 'berm' or hill to keep the wind out.

Livability

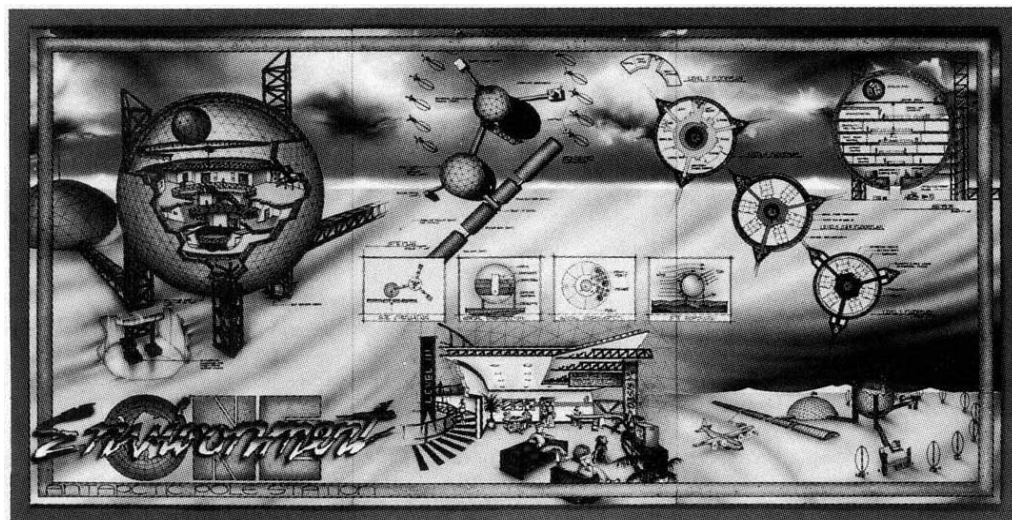
Surveys of South Pole veterans have shown that station designs should allow equally for common space, to allow residents to congregate, and physically separated areas, so that

people can get away when they wish. Many of the designs use walkways to separate areas of day-to-day activities such as eating, sleeping and working, to create the impression that one is not spending one's entire life in the same place.

A view of the outdoors (such as it is — the landscape at the Pole is utterly flat and barren) is also considered important. One advantage of chains of small buildings, rather than large central structures such as domes, is that they allow everyone to have a window.

Power and heat

The South Pole is one place where solar power needs no sell-



Better than sheds — one of the student designs for the new station.

ing. At an altitude of 2,835 metres, the atmosphere is thin enough to allow for intense sunlight in the summer months. For almost a third of the year, the South Pole is the sunniest place on Earth. However, the rays arrive at nearly horizontal angles, making rooftop solar panel almost useless. Although boxy, vertical-sided structures have little of the dome's aesthetic appeal, they are far better at collecting solar heat. Wayne Tobiasson, director of the CRREL project, believes that air-filled solar collectors on vertical walls could heat the station virtually on their own in the summer months.

Some of the students proposed wind turbines to provide electric power all the year round. But Tobiasson says practical concerns may prohibit such innovations, despite the temptation of nearly constant winds at the Pole. "We've looked at wind power, and it's appealing", he says. "But it's tough to make mechanical devices that can spin at 100 degrees below zero."

Science

Research at the South Pole generally falls into three broad categories: atmospheric, astronomical and geological. Each science category requires environmental conditions that are often incompatible with other normal activities at the station.

Most design proposals assign each field of research a special 'sector' where it would be isolated from interference. The Metcalf and Eddy design, for example, features an 'upwind sector' for atmospheric studies that require absolutely clean air, a 'dark sector' for astronomy, a 'quiet sector' for geological studies that might be disturbed by vibrations, and a 'downwind sector' for balloon launches.

Safety

The South Pole, with less than two inches of precipitation each year, is the world's driest place. Dry air and a lack of liquid water makes fire a constant threat. Most of the proposals would physically separate buildings to keep any fire from spreading to the entire station.

Aesthetics

The geodesic dome at the Pole was originally selected partly for its aesthetic appeal, a decision some NSF officials soon began to regret as construction of the complex structure took a year longer than planned. Nevertheless, NSF still believes that an attractive and thoughtful structure should symbolize the human presence at the Pole.

The agency is willing to make some practical concessions, on matters such as cost and construction complexity, for appearance. But function still dominates form on the Pole. "You can talk about the poetics of architecture until the cows come home", says AIA competition coordinator Kevin McGillicuddy, "but unless it keeps the cold out, it won't work."

Christopher Anderson

Coping with it all

Palmer Station

"DID you see the guy in the dress?" Antarctic veterans like to ask visitors these kind of questions. It makes the newcomers blush, especially when the answer is yes (although, in this particular case the "dress" turned out to be more of a simple tan robe with a sash, worn over bare legs and tennis shoes). Compared to average city dwellers, the residents of Antarctica lean towards the extremes, and they know it. The kind of person who signs up for nine months of darkness, sub-zero temperatures and near-isolation is not cut from the standard cloth.

Polar psychology has fascinated researchers for decades. In the 1960s and 1970s, psychologists studied thousands of enlisted Navy personnel who overwintered in Antarctica to see how they handled stress. The National Aeronautics and Space Administration (NASA) has conducted studies on Antarctic researchers and support crews to learn more about behaviour in space-like conditions. Navy psychologists examine overwintering residents to learn how to exclude future candidates who may not be able to cope with the pressure.

Despite all this research, psychologists are hard pressed to define the polar 'type', at least when it comes to specifics. "Basically it's the sort of person who doesn't get too many highs and doesn't get too many lows", says Frederic Glowgower, chairman of the psychology department at the Navy Hospital of San Diego, where he runs the Navy's polar screening process. "We're looking for people who can keep themselves amused with a lot of books and movies."

Glowgower and several other Navy psychologists interview all overwinter Antarctic applicants to spot those who may have trouble with polar life. They look for an ability to handle stress, people and regulations, of which there are many, given the risks of winter conditions.

They have good reason to be concerned, for problem cases are not uncommon. Every year some researchers or support crew leave early, when they find they can no longer stand the life. Depression is common, as is alcohol abuse. Liquor is rationed and closely monitored at US stations. McMurdo base offers racks of brochures on how to control anger and depression. And sometimes even the precautions are not enough. The doctor at one Argentine base burned his own station down. And a British team member apparently killed himself by one day walking out onto the ice, never to return.

Between 10 and 25 per cent of the US applicants are eliminated in the psychological screening. Unlike Antarctic residents from some other countries, US applicants who are escaping difficult situations at home are not encouraged. "If you're someone whose life circumstances are hanging from a thread, that's a problem", Glowgower says. Simi-

larly, people who value their privacy are told to expect the worst. Gossip travels fast in cramped surroundings.

Loners, on the other hand, often do well. People who do not depend on a large social network are not likely to be disappointed when they realize that they are going to spend the next nine months with a dozen companions. (In Antarctic tradition, even being a loner is sometimes taken to the extreme. One particularly reclusive resident of McMurdo base prompted the printing of special T-shirts to be issued on rare occasions. They read: "I saw [his name], the Mysterious Man of McMurdo".)

Given the stress of Antarctic life, a winter-over would seem likely to have lasting psychological consequences. That turns out to be true, says University of California San Diego psychologist Lawrence Polinkas, but not in the way one might expect.

Polinkas studied the 20-year-old records of 327 Navy personnel who overwintered in Antarctica and compared them with 2,396 personnel from the same period who also passed the psychological screen, but for various reasons did not actually overwinter. He followed the subsequent medical histories of the two groups, and discovered that the people who actually spent a winter in Antarctica had significantly fewer hospitalizations for tumours, disorders of the endocrine, nervous and metabolic systems, and for back problems and other musculoskeletal diseases.

"It's called stress inoculation, Polinkas says. "When individuals go through uniform stressful situations together, they seem to become better at handling stress in general." He says winter-over veterans report increased feelings of autonomy and self-reliance. "They think 'If I can handle this, I can handle anything'."

Polinkas also found an increased use of avoidance and denial to handle stress among those who overwintered. Head-in-the-sand coping methods may not seem very practical, but "in Antarctica, where you can't always escape the stress, there may be no alternative", he says. "It teaches them to cope with limited resources. What would be considered maladaptive in our society may be adaptive on the ice."

At Palmer Station, some coping mechanisms are more obvious. A visitor is quickly briefed on the rules: "You can talk about yourself, but not other people. And respect privacy." Among the 50 researcher and support staff at Palmer there has developed a unique descriptor of one's psychological state: toast. Residents toast bread to a darkness appropriate to their mood. They hang the symbol on their doorknobs. The blacker the bread, the more eager they are to leave Antarctica. Says one: "When all I have is a mound of charcoal at the foot of my door, I'm out of here. I'm toast."

C.A.

TOURISM

Let sleeping dogs lie

Palmer Station

THE trouble with Antarctic tourists is that there are not enough of them. And they are too well behaved. If tourists were swarming the rocky shores of Antarctica, poking at penguins and leaving film wrappers behind, there would no doubt be world outrage and calls for restrictions. But that is not the case, and the National Science Foundation (NSF), which runs the US programme, is left in a quandary. The number of visitors is still manageable — fewer than 2,000 last year — and reports of serious abuses are virtually nonexistent. That could change, but until it does, NSF is hesitant to jump the gun with draconian regulations.

"The problem", says Polly Penhale, Antarctic programme officer at the NSF, "is mostly potential." Tour companies now work closely — and voluntarily — with NSF to coordinate visits, ensuring that the ships neither pile up at Palmer Station nor over-visit the most popular penguin rookeries. Relations have become so chummy that Penhale, who gives lectures to the tourists when she is at Palmer, has become internationally known for the cakes she serves the visitors.

The cruise ships usually have trained scientists on board to give lectures and to make sure that tourists obey the laws of the Antarctic Treaty — do not approach animals, do not take anything, do not leave anything, and so on. But the tourists seem to need few reminders. With an average of some \$8,000 per person invested in the trip,

they tend to be well informed, scientifically and ecologically literate and respectful of the continent.

"So far, so good", says Penhale. Her worry is there may eventually be less responsible operators. NSF's policy is neither to encourage nor discourage tourism. Its attempt, in 1989, to ban tourism at US stations provoked complaints from spurned visitors, who argued that they had a right to see the way their tax money was being spent, which forced the NSF to reverse its decision.

Beyond environmental issues, there is a question of whether the United States is

New Zealand DC-10 that hit a mountain in 1979, the US court of appeals ruled that the United States did have a legal responsibility in Antarctica (although the court did not find negligence in that particular case). But in a subsequent Californian decision, a district judge ruled that the United States could not be held liable for the deaths of two US contractors who fell down a crevasse.

"It's an open legal issue", says Lawrence Rudolph, deputy general counsel at NSF. Rather than fight lawsuits case by case in the future, NSF has asked the Justice Department to clarify the Tort Claims Act to include Antarctica as a 'foreign country', exempt from suits. Meanwhile, many NSF contractors have taken out personal liability insurance, for fear that they themselves will be sued even if the government cannot be.

So, without a clear policy on tourism and an unclear legal position, NSF gingerly waits to see what will happen next. If visitors to the Antarctic stay at the level of several thousand each year and tour operators continue to work with government officials to minimize the effect on the environment, restrictions may not be necessary. But the lessons of the *Bahia Paraíso* are still a fresh warning of where things can go wrong. When the Argentine supply ship went aground in 1989 with 85 tourists aboard, it happened to be less than a mile from Palmer, whose staff were able to rescue everybody on board.

Had it been just a few miles further away, the situation might have been very different. When the inevitable next accident happens, the future of Antarctic tourism may hang in the balance.

C.A

Wish you were here? The lucky survivors from the Bahia Paraíso arrive at Palmer Station. (Photo: NSF/Ted Delaco)

liable for damage suffered by tourists. Although the US Tort Claims Act offers the government protection from suits for damage suffered in foreign countries, Antarctica is not considered a country and is not subject to that exemption. Legal precedent, meanwhile, is clouded by two contradictory court decisions. In one case, brought by the families of some of 257 victims of the Air

ANTARCTIC researchers, like any isolated group, have developed their own home-brewed vocabulary, for all the usual reasons as well as to spot the 'fingies' (see below). The derivations of the following Antarctic idioms are speculative at best, and their meanings tend to change somewhat with time, but the list can be considered relatively up to date.

When they say... They mean....

BEAKER	Scientist (as opposed to support personnel).
BLACK TIE	No NSF-issue red clothes.
FINGIE	[Unprintable] New Guy on Ice, FNGI.
THE ICE	Antarctica.
HEY, IT'S A HARSH CONTINENT	Stop griping.
DIAPERS	Unpopular NSF-issue flotation coat with flaps that button-up between the legs.
ANTARCTIC 10	A person of the opposite sex who might be considered a "5" elsewhere.
BUNNY BOOTS	Huge inflatable boots that are reported to explode on airplanes if not deflated.
SKUA	Frozen chicken, an Antarctic staple.
SLIDERS	Frozen hamburgers, another Antarctic staple.
CHICKEN COOKIES	Frozen chicken patties, sometimes mistaken momentarily for dessert.

Talk like a beaker



CHINESE LANDING A phonetic pun, based on the usual aircraft angle when landing in stiff Antarctic cross winds: one wing low.

HOLLYWOOD SHOWER A Navy term, derisively used to describe showers of longer than the allotted two minutes.

BAG DRAG The routine of carrying one's NSF-issue bag of polar clothing to the airstrip, only to find that the notoriously unpredictable Antarctic weather has once again halted all flights for 24 hours.

COUNTRY MICE Scientists and their assistants who get to travel to camps around Antarctica.

CITY MICE Support personnel whose duties force them to remain at McMurdo Station.

HOUSE MICE Personnel on periodic janitorial duty.

When things go wrong

Aboard the *Polar Duke*

To be a biologist studying the ocean food web in Antarctica, it helps to know how to weld. Underwater monitoring stations must be attached to all the scrap iron in sight to keep them from being dragged away by icebergs. Some experience in sonar electronics is also handy, as is a familiarity with satellite navigation, especially when trying to find a station that has spent a year under water. And when the devices will not respond to acoustic release commands, a thorough knowledge of salvage techniques is essential, including a working familiarity with a grappling hook.

Those are the lessons from one ill-fated recovery exercise, an all-too-typical example of how things can go wrong in Antarctica.

David Karl is studying the carbon cycle in the Antarctic, from the algae and krill down to the sediment. Just how much of the carbon-based life near the surface of the Southern Oceans actually reaches the sediment is one of the great unknowns of the climate change equation. To explore the particular question, the University of Hawaii molecular biologist placed several sediment traps — essentially upside-down funnels with collection jars at the bottom — at various sites near the Antarctic Peninsula to record the amount of living matter that falls through the water column in a year.

Dropping the sediment traps was easy — Karl and his colleagues wrapped an old boiler with 15 metres of iron-link chain, tied it to cable on which two traps and some floats were attached, and threw it overboard.

That was last year. This February it was time to recover the traps and see what they had caught. The National Science Foundation icebreaker *Polar Duke* scheduled a brief detour to pick up the traps on its way from King George Island down the peninsula to Palmer Station.

Modern traps — indeed, most modern

underwater recoverable objects — are designed to be released remotely, by sending a special acoustic signal. The traps (actually electronics in the mooring to which the traps are attached) put out a weak locator signal so that one can place an acoustic transducer — essentially an underwater loudspeaker — near enough to be heard, but not so near that the whole thing pops up under the boat.

But when the *Polar Duke* reached the area where the traps were supposed to be, Karl could not pick up the locator signal. He sent the release signal anyway, in the hope that it was just the transmission portion of the trap's electronics that had failed, but the icy waters remained unbroken.

At this point, one might have been tempted to give up. The only way to recover a trap that will not come up on its own is to drag for it with a grappling hook, a century-old technique that basically means putting a lethal-looking iron hook overboard and cruising back and forth until it snags something. (Things go wrong so routinely in the Antarctic that there has even emerged a term — 'plan Z' — to describe the necessary use of methods so far-fetched that they would be ridiculed elsewhere.)

Normally, the odds of the hook working are astronomical. It is, after all, a big ocean.

But Karl still had some technology up his sleeve. When he had dropped the traps a year earlier, he had recorded their position with the use of the Global Positioning System (GPS), the worldwide satellite network that the US Department of Defense has set up to (among other things) guide cruise missiles.

Without a special military decoder, GPS coordinates are accurate only to several tens of metres. But even that is a great leap up from map readings or celestial bearings. With GPS as his guide, Karl decided he could get close enough to the traps (assuming they had not moved) to raise the odds of snagging them to an acceptable risk.

That, however, would have to wait until the *Polar Duke* returned, nearly a week later. Early one morning on the return voyage, the *Polar Duke* arrived again at the site, with a grappling apparatus ready to be deployed. The hook (a home-made three-metre bar with four three-prong barbs on chains, adorned with the stencilled words 'Claws-o-Death') was lowered into the water shortly before noon.

After less than an hour's searching, the *Polar Duke's* sonar recorded a series of objects vertically separated by some 30 metres — the acoustic profile of the sediment traps. Within seconds the winch operator also reported tension on the grappling line and the icebreaker slowed to a stop.

The winch slowly pulled up the hooks as Karl and the crew of the *Polar Duke* leaned over the rail searching for a glimpse of the yellow floats of the traps. Several long minutes later, the floats surface was 100 metres behind the ship, to cheers.

After another 30 metres of cable was winched in, the upper trap surfaces and has hoisted above the stern deck of the icebreaker. The researchers surrounded it, examining the 13 collection jars on its rotating carousel. All but one of the jars were virtually empty — more proof that this part of the Antarctic Ocean has experienced no algae bloom this year, something researchers elsewhere had already observed.

The crew turns back to the cable still stretched over the edge. One hundred metres below hung the second trap, and 100 metres below that, damaged the boiler and chains, encrusted with over a ton of silt from a year under water. Observers on the upper deck, watching the tension in the stressed wire, moved back, lest it break and whip towards them.

The winch was started again, slowly, and the cable again began to reel in. But by now the stress dynamics of the cable had changed. As the cable shortened, it also became less elastic, and with every pitch of the ship, the weight below pulled it to its breaking point. Finally, with a groan, the wire parted, and the weights, the second trap, and 200 metres of cable plummeted to the ocean bottom.

An effort was made to drag again, this time on the ocean floor, but after a frustrating hour, the Claws-o-Death was reeled back in, beaten by underwater rocks into twisted junk. Karl gave up.

"The important thing is the data", he says. The traps cost \$16,000 each, but the sediment record they hold is worth far more. Data from the upper trap will answer the most pressing questions about sediment in the region, but the loss of the lower trap is a disappointment. Frustrating, too, is the loss of the mooring, which was the cause of all the trouble. Karl will be back again to drop other sediment traps, but without knowing what caused this one to fail, he has to eventually expect another afternoon with the grappling hook.

C.A.

In troubled waters

Palmer Station

In January 1989, shortly after the Argentine supply ship *Bahia Paraíso* hit the rocks and spilled 250,000 gallons of diesel fuel into the water near Palmer Station, something happened to all the skua chicks nearby: they died. That much is sure.

But whether it was the effect of fuel oil on the colony that killed the fledgling seabirds, or simply malnutrition, remains the subject of a long-running scientific battle, pitching two graduate students against members of the avian biology establishment. As one of the only examples of a wildlife population that was extensively studied both before and after an oil spill, the case of the skuas is hardly academic. A resolution of the dispute may shed light on the ability of natural populations to recover from unnatural disasters, and suggest priorities in handling the environmental effects of future spills.

In the upstart corner are Zoe Eppley and Margaret Rubega, who were studying skuas around Palmer at the time of the spill and who are now finishing their graduate degrees at the University of California at Irvine. They argue that the hundreds of skua chicks that perished after the spill were killed by other skuas when their oil-weakened parents neglected the nests.

Opposing them are Wayne Trivelpiece and William Fraser, two long-time avian biologists who are now with the Old Dominion

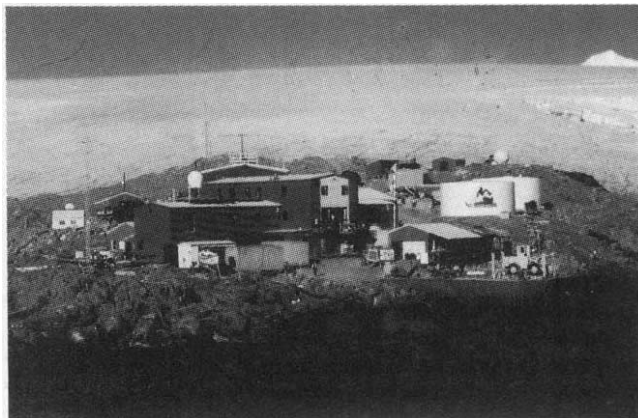
University Polar Oceans Research Group after a long tenure at the Point Reyes Bird Observatory. Trivelpiece and Fraser say that their studies of skua chicks show that the Palmer birds were 'starving' at the time of the spill, and would have died anyway.

The dispute, fought out in the pages of *Nature* (340, 513; 1989 & 345, 211; 1990) and in a *Science* news article last summer, seemed impossible to reconcile. Trivelpiece and Fraser, using data from several skua colonies in the same summer, said that poor nest attendance is not uncommon among skua adults, especially during food shortages of the sort they observed that year. In the *Science* article (249, 243; 1990), Trivelpiece was quoted as saying "by 21 days, their birds are 600 grams and ours are 1,100. They're obviously starving."

Faced with such a challenge, Eppley went to the books. She found that previous studies on skua growth showed weights at three weeks of between 470 and about 700 grams for chicks that eventually survived to adult-

hood. Her chicks had averaged somewhat over 500 grams at that age — on the light side of the spectrum, but probably not starving. "True, they weren't growing like gangbusters, but there are plenty of instances of birds that weighed what my birds did, and went on to survive", she says.

Still, that does not explain how Trivelpiece's birds could have weighed nearly twice as much as the same age. At the request of a reporter, Trivelpiece reexamined his



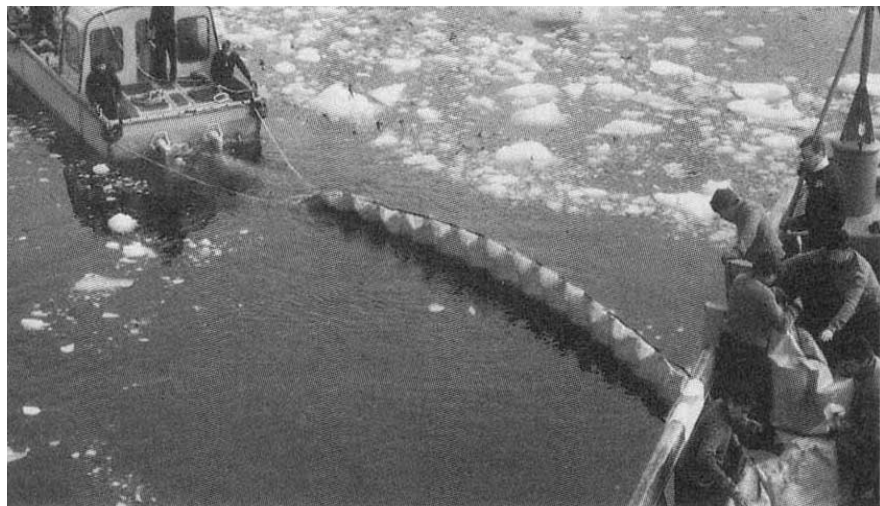
Palmer Station: a contrast to new buildings at McMurdo (page 299).

data. He found that the 1,100 gram weight was actually the average weight for 30-day chicks; average 21-day chicks weighed between 750 and 785 grams in his studies — at the other end of the spectrum from Eppley's 500 gram chicks, but no longer unbelievably so. The error had cropped up as a misunderstanding in a telephone interview with the *Science* reporter, he says.

An argument over the effect of weather on the skuas seems to be going the same way. Fraser believes that a storm, which occurred soon after the oil spill, was responsible for many of the deaths, both at Palmer and at Trivelpiece's colony on King George Island. Eppley has also investigated that possibility. Using meteorological records, she found that storms usually do correlate with the deaths of skua chicks.

But in the 1989 season, records show that the suspect squall struck after the chicks were already dead. "I think Bill is going with his memory on that one", she says.

The bottom line? Trivelpiece still says the Palmer skua chicks were starving and Eppley continues to maintain that they would have lived had not oil forced their parents to neglect the nests. But with the data converging, the argument now appears to be a matter of interpretation, rather than disputed facts. Eppley suggests that the difference in chick weights at Palmer and King George Island, 250 miles away, is likely to be more a function of the varying conditions and bird types at the two sites than an indication of serious malnutrition at Palmer. It is likely that the Palmer skuas were not getting as much food as they should, she agrees. But it was still the oil that pushed the colony over the edge.



Upside-down in the shallow waters near Palmer Station, the *Bahia Paraíso* can be seen at low tide, a reminder of an ever present environmental threat. The Argentine supply ship, which grounded and capsized in an accident two years ago, still holds some 70,000 gallons of fuel oil. Should the ship break up in a storm, that oil could be released, soaking hundreds of penguins and skuas as it did in 1989.

Debt-ridden Argentina cannot afford to salvage the vessel, but the country has taken the responsibility to do what it can. Last month, the Argentine Navy and Palmer personnel staged the first joint oil-spill drill in Antarctica — a full-speed simulation of the planned response to a severe oil leak at the wreck. Crews from Palmer and an Argentine warship encircled the exposed hull with floating containment booms and practised deploying absorbent materials and a small oil skimmer. US oil-spill experts monitored the operation.

Although NSF had hoped to have the wreck contained within an hour or two, practice showed that an Antarctic spill response is more difficult than similar operations elsewhere. Crews found that even small icebergs tend to drag the booms with them, and much of the time was spent fighting off threatening ice floes with pikes and small Zodiac inflatable boats.

Penguins losing the struggle?

King George Island

WAYNE Trivelpiece boarded the Antarctic icebreaker *Polar Duke* last month with bad news. For the first time in 13 years, the biologist, who studies penguins on a desolate island beach off the Antarctic Peninsula, had recorded a fall in the local population — some 20 per cent of the 18,000 penguin breeding pairs that normally return to the rookery had failed to do so.

Worse still, the missing included large numbers of both Adélies and chinstraps, the two main species in the region. New research by biologist William Fraser, Trivelpiece's colleague at the Old Dominion University Polar Oceans Research Group, has shown that natural fluctuations in sea-ice coverage are often the major factor in penguin survival and breeding success. But because the winter feeding strategies of the Adélie and the chinstrap are so different, declines in one species have always been offset by increases in the other. To lose 20 per cent of both species in the same year, Trivelpiece says, is unusual. To have this occur for the last three consecutive years is unprecedented, and may suggest a shortage of krill, the shrimp-like crustacean that comprises most of a penguin diet.

Normally, returning penguin populations can be correlated to the weather conditions of the previous winter. In a cold winter, there is more sea ice, which favours the survival of the Adélie penguin, a species that winters under the pack ice. During a mild winter, on the other hand, the chinstrap penguin has the advantage. It feeds in open water, and the absence of sea ice means better hunting close to home.

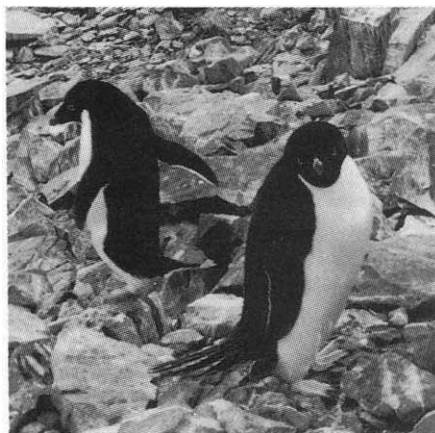
The years 1986 and 1987 had harsh winters; from 1988 to 1990, the winters were mild. Ordinarily that would have meant depressed chinstrap populations for the first two years, then a change of fortunes as the Adélies suffered over the next three winters.

Instead, Trivelpiece has seen a 10–20 per cent decline in both species for all of the past three years.

Until this year, Trivelpiece was willing to explain away the anomalous chinstrap declines as a statistical aberration. Now, he says, after another mild winter, "we knew it wasn't just a fluke".

Although Trivelpiece says that krill shortages are just one possible explanation for the decline, he notes that the change in the population patterns corresponds closely with an increase in krill harvests. From 1982 to 1985, fishing fleets took between 130,000 and 200,000 tons of krill from the Southern Oceans each year. But in 1986, they doubled their take to nearly 400,000 tons. Two years and two harsh winters later, penguin populations began to decline.

If, indeed, a krill shortage turns out to be the problem, one obvious suspect is fishing. Japanese and Soviet trawlers fish mostly from the region where Trivelpiece believes



Adélies — 10 per cent population drop three years running.

his penguins feed. An international body created by the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) is supposed to watch for overfishing, but counting krill is a difficult and inexact science (see page 296). Krill tend to move in huge swarms, which may easily be missed in random sampling of the Southern Oceans.

Biologists such as Trivelpiece and Fraser believe that penguins and other predators are better at finding krill than we are. So why not let the predators do the counting for us? If the predators cannot find krill, they starve. Track predator populations, the biologists propose, and you will have an accurate measure of krill populations. That is what Trivelpiece, his co-investigator and wife Susan, and other colleagues do each summer on their bleak beach on King George Island. And if penguin populations are any guide,

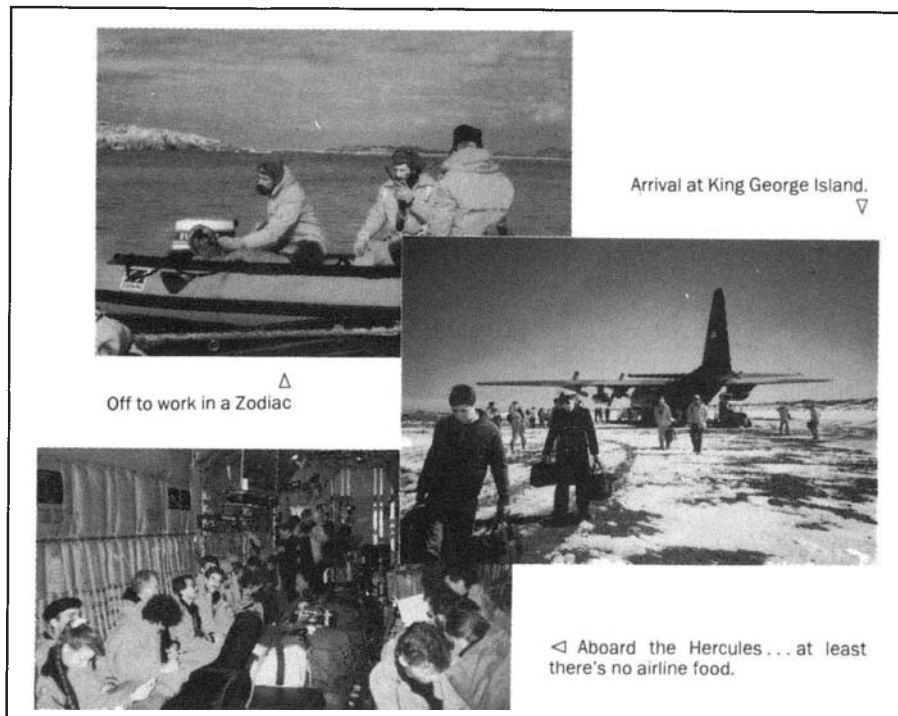
the krill are becoming hard to find.

But if overfishing is to blame, and 400,000 tons of harvested krill a year is too much, why did it take several years for the effects to show? The answer may lie with the krill themselves. New data suggest that the two-inch crustacean may live and breed far longer than anyone had previously suspected. If the average krill lifespan were just a year or two, overfishing would quickly show its mark. But researchers now believe that krill may naturally live 6 to 8 years. It is not a new population of krill that the fishing fleets thin each year, but mostly the same population as the year before. Yearly fishing totals now seem less important than the cumulative krill harvest over the decade — some 3 million tons and counting.

What that means for krill fishing — and for krill — is still open to debate. CCAMLR has not finished compiling krill populations figures for this year, but previous years' counts (taken mostly with sonar surveys and nets) showed no obvious decline. Those counts, however, are based on poor statistics and spotting sampling. Fishing nations have argued that the data are too unreliable to be used as the basis for krill restrictions — an argument that seems sure to be renewed in the light of the findings.

While no one is likely to restrict fishing on the basis of penguin populations alone, the worrying population trend at the beach cannot be ignored, researchers argue. Further studies on krill life cycles and improved sonar surveying techniques may help to refine the counting techniques. But if portions of the Antarctic waters are indeed losing their krill, CCAMLR — not to mention the penguins, whales and other predators that depend on the krill — may not be able to wait.

C.A.



Arrival at King George Island.

Off to work in a Zodiac

◁ Aboard the Hercules... at least there's no airline food.

Green issues fuel funding

Palmer Station

If the case of the US Antarctic programme is any guide, the principal effect of greenhouse warming and ultraviolet radiation may be wild, unpredictable growth. Riding the coat-tails of international concern over ozone and global change, the US Antarctic effort has unexpectedly found itself at the focus of the decade's hottest environmental research, a windfall that has resulted in a tripling of funding over the past ten years.

Since 1980, the National Science Foundation (NSF) programme has seen its Antarctic budget increase from \$55 million to \$175 million, a spurt in growth that will have an immediate impact in a host of new facilities and improvements at the main US bases.

At McMurdo Station, 750 miles from the South Pole on the rocky shores of the Ross Sea, a 46,000-square-foot, three-storey science laboratory is nearly finished. It will join newly renovated dormitories and administrative buildings to finish a five-year renaissance for the 1,200-person base, until recently best known for its mud and hard life.

The South Pole's Amundsen-Scott Station is also seeing a flurry of construction. Twenty years of drifting snow have half-buried the 50-foot aluminium geodesic dome that houses most of the station, causing uneven pressures and structural damage. This year, engineers bulldozed away tons of snow, repaired parts of the dome and raised some parts by nearly a foot to level the structure and balance its loads. They believe that the repairs have extended the dome's life span by some 20 years. Work will begin later this year on a new summer base at the South Pole, to house the increasing numbers of scientists who now come to the already overcrowded station during the peak research months. And later in the decade, NSF hopes to begin construction on a new and much larger station at the Pole (see page 289).

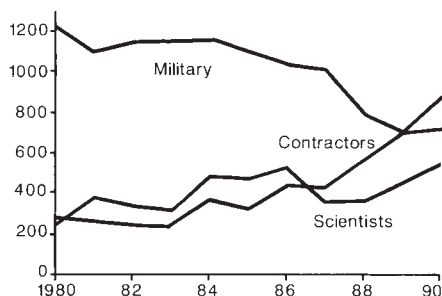
At Palmer Station, on the relatively temperate Antarctic Peninsula, NSF is getting ready to start its first Antarctic Long-Term Ecological Research (LTER) site. Under a six-year grant, researchers at Palmer will carry out studies throughout the food web — from ocean dynamics and algae growth to penguins — to try to understand how the 'carbon cycle' biology of the Antarctic oceans may affect global warming, and vice versa.

One Antarctic 'facility' that is not yet in the polar region is the *Nathaniel B. Palmer*, a major new research vessel with ice-breaking capacity now under construction in Louisiana. When the 300 foot, \$83.8-million vessel is first launched around the end of the year, it will join the 219-foot *Polar Duke* in full-time Antarctic service to complete NSF's two-ship research fleet.

NSF has no plans for further major construction once these facilities are finished. Although the Antarctic programme is ex-

pected to continue growing, agency officials intend to increase research efforts not by building more structures, but by bringing in more scientists and fewer support personnel. Already an NSF 'streamlining plan' has doubled the ratio of researchers to support staff in ten years by hiring private contractors to take over any support function's from the Navy, which is notorious for its bureaucratic inefficiency and redundancy.

"The flexibility of using contractors is that we don't have to carry them when we don't need them", says one NSF official. "Using



Scientists and contractors are an increasing proportion of the US contingent.

fewer people saves money, as well as wear and tear on the continent."

Another way to get more science without importing more manpower is to use automation, a trend that has come far since its beginnings in weather-monitoring stations. Automated clean-air facilities, telescopes, seismic stations, ozone-monitoring equipment and underwater sampling stations are all in use, overseen by the technicians who maintain other station equipment, or by no one at all. Scarce Antarctic slots can now be filled by other researchers whose work requires personal attention, leaving the most mindless research responsibilities to the machines.

C.A.

Pole position

Washington

US researchers will build three new telescopes at the South Pole, as part of a new centre announced last month by the NSF. The facility will be one of the 14 new research centres funded by NSF as the second phase of its three-year-old network of interdisciplinary, university-based Science and Technology Centers (STCs). Headed by astronomers from the University of Chicago, the South Pole centre will operate as a consortium, with eight university and industry partners. NSF funding will be \$2 million for 1991, and up to \$13.6 million over the next five years.

Each of the three South Pole telescopes will focus on a fundamental problem in astronomy. The Cosmic Background Radiation Anisotropy (COBRA) Project will look for tiny (one part in a million) variations in the cosmic radiation background as part of the investigation of the origins of galaxies. A second telescope, known as the South Pole Infrared Explorer project (SPIREX), will take advantage of the South Pole's low infrared background to study the formation of stars in young galaxies.

The third project will focus on the interstellar medium in nearby galaxies. Known as the Antarctic Submillimeter Telescope/Remote Observatory (AST/RO), the project will also measure ozone in the Earth's atmosphere.

Project members plan to operate the telescopes during the harshest winter months at the Pole, when the station is isolated from the outside world and temperatures can drop to -100°F .

Developing telescopes to operate in those conditions, and to follow celestial objects from a constantly moving surface, will require new technology that will help in the design of even more advanced telescopes elsewhere, centre officials say.

C.A.



Nearly finished, in this October 1990 view, the new science laboratory at McMurdo Station. Cold Regions Research Laboratory photo by Wayne Tobiasson.

The trouble with trawlers

Washington

PRACTICALLY everything in Antarctica dines on krill. Billions of the shrimp-like creatures are the big middle link of what is essentially a three-part food chain — algae, the krill, then nearly everything else, from whales to penguins. Even humans eat krill: fishing fleets from six countries netted some 400,000 tons of the two-inch crustaceans in the Antarctic oceans last year.

They could have taken even more. What kept those countries, and others, from sending bigger fleets and keeping them in Antarctica longer to net krill is simple economics — they find it hard to sell. Krill are expensive to harvest, turn an unappetizing algae-tinted green during the height of their feeding season and are barely big enough to lift with a fork. But that may change. Krill will not get bigger, but the human appetite for them may.

So far, man's impact has been minimal — krill overfishing, to the extent that it has even been observed (and even that is debated, see page 294), appears to have been mostly limited to small areas that soon recovered. If the market improves, however, there are at present no limits to the amount of krill that could be taken. And as the krill go, so do all the Antarctic animals that feed on them.

"What if the world suddenly decides it loves krill and wants them in their burgers?", asks Raymond Arnaudo, head of the US Department of State's division of polar affairs. "We could be looking at some real difficulties, not just for the krill, but for the predators."

Arnaudo, fortunately, is in a position to do something about it. He is the US delegate to the commission established to give effect to the Convention of the Conservation of Antarctic Marine Living Resources (CCAMLR), the nine-year-old fishing agreement signed by the members of the Antarctic Treaty. A precaution against any repetition of the excessive whaling and sealing that characterized much of the past century, CCAMLR was created to make sure

that krill, fish and marine mammals in the Antarctic are never again overfished.

But that, at least for krill, has turned out to be a surprisingly difficult assignment. CCAMLR is supposed to watch for unexpected changes in krill population, and then to regulate fishing if it seems to be starting to shift the balance of nature. Spotting population decline requires at least two sets of numbers: the baseline 'normal' population, and subsequent estimates. Regrettably, CCAMLR has neither.

The problem, explains Robert Hofman, scientific director of the US Marine Mammal Commission, is that counting krill is no small task. Krill tend to swarm by the millions in a small area, and to be entirely absent elsewhere. Researchers still have virtually no idea what drives this behaviour, or how to predict where it might happen next.

All the direct methods now used to count krill — nets, sonar and other hydroacoustic techniques — require that the observing vessel is almost on top of them. Miss the swarm by half a mile and one may find no krill at all. "It's frustrating. The distribution is very patchy," notes Hofman.

It would be prohibitively costly, if not impossible, to try to monitor each and every krill predator that might be affected by harvesting krill. Therefore treaty partners have begun to set up a network of 23 stations at which researchers will monitor populations of land-based predators such as penguins, fur and crabeater seals, albatross and petrels.

But indirect measurement have their own disadvantages. Uncertainty in the natural fluctuation of both the predators and the krill populations makes correlation difficult. Even for well-studied species such as penguins, researchers are only now beginning to understand the most basic factors (such as sea-ice coverage) that determine year-to-year population changes. Long-term research on the dynamics of the entire Antarctic food web, from krill up, may be required before indirect measurements can be trusted

to reveal krill populations. And although such an effort is under way, the programme at this point is, as one official puts it, "mostly conceptual".

Yet CCAMLR must make decisions now, before the fishery expands. "The question becomes how to deal with uncertainty", says Hofman. Uncertainty requires a healthy dose of precaution, he believes.

Other US officials agree. As a precaution the United States and several other CCAMLR treaty partners have proposed setting some limits on krill fishing. But Japan and the Soviet Union argue that there is no evidence of a problem and no limits are necessary. The two nations — whose trawlers take most of the yearly krill harvest — say that they have no plans to increase their catches.

The krill market could change, they acknowledge, and they make no promises for the future. But they point out that estimates of total krill population are in the 100-million-metric-ton range. Some 10 million metric tons of that could be harvested each year without endangering overall populations, according to one estimate. Current krill harvests have averaged only around 400,000 tons annually for the last several years. How could such drop-in-the-bucket fishing be a problem?

Worldwide, it probably will not be, Arnaudo says. But on a local scale, krill fishing can have more serious implications, especially for predators. No one knows how far penguins, for example, can travel for food in winter. If their range is relatively limited, over-fishing of even a small area could decimate the penguin colonies that feed there. There is currently nothing to stop fishing fleets from exhausting an area if they find it convenient to do so. And without better data on the risks of such fishing, it will be difficult to impose restrictions.

Nevertheless, at the next CCAMLR meeting, to be held in October 1992 in Hobart, Tasmania, the United States will make its strongest push yet for restrictions. Arnaudo says that the US delegation will attempt to encourage the Soviets and Japanese to agree to some limits before the meeting. Meanwhile, delegations from several countries will argue with their own governments for more money for monitoring. "The real problem is that it's a vast area to monitor with a minuscule budget", he says (the United States put a little over \$1 million into the programme last year). And until the research programme matures and the population monitoring improves, advocates of krill limits will continue to find stiff resistance to soft data.

C.A.

■ For an Australian view on the krill conservation issue, see page 306.

Thanks to Jeffrey Norris and Winifred Reuning at the National Science Foundation, Robert Hofman of the Marine Mammal Commission, Wayne and Susan Trivelpiece of Old Dominion University, Kevin McGillicuddy of the American Institute of Architects, and Sherburne Abbott of the Polar Research Board for their invaluable assistance.

Playing according to the game plan

Cambridge

"We are the envy of most Antarctic nations", asserts Barry Heywood, deputy director of the British Antarctic Survey (BAS). Heywood's brash self-confidence, a rarity among contemporary British scientists, stems from two linked developments that transformed the fortunes of BAS during the 1980s. First, BAS's annual budget grew in real terms by more than 60 per cent between 1982-83 and 1990-91, when it reached £16.3 million. Second, the British scientific presence in the Antarctic has become perhaps the best co-ordinated of any of the Antarctic nations, centred around a clear long-term strategy.

The turning-point for BAS, after a decade of neglect in the 1970s, came with the Falklands conflict of 1982. Investment in the South Atlantic and Antarctic suddenly became a political priority, once British forces had regained the islands from Argentina. In 1983, the government approved plans put forward by Sir Hermann Bondi, then chairman of the Natural Environment Research Council (NERC), to expand British Antarctic research. Bondi's original plans, refined and extended, form the backbone of a glossy NERC document of 1989, *Antarctica 2000*, which defines the direction of British Antarctic science over the next decade.

To match BAS's activities to the developing NERC strategy, BAS was restructured in 1987 into six new science divisions: Ice and Climate, Geology, Geophysics, Terrestrial and Freshwater Life Sciences, Marine Life Sciences, and Upper Atmospheric Sciences. The new structure (which replaced the traditional academic division into atmospheric, Earth and life sciences) brings together researchers, often from different disciplines, to focus on the themes laid down in the NERC strategy.

This approach is best evident in the ice and climate division, which has united glaciologists and atmospheric scientists to provide data vital to modellers attempting to predict the pattern of climate change. For example, BAS scientists are now measuring the transport of water vapour into the atmosphere over the Antarctic, and its deposition as snow on the continent. The data will be used to refine the UK Meteorological Office's global climate model.

The centralization of British Antarctic research around the NERC strategy and BAS was completed with the opening of a new and larger headquarters building at BAS's Cambridge site in 1989. Staff have now been moved into the building from BAS's former outposts all over the country. The end result is a coherent research effort, Heywood says, which benefits from having the logistics of conducting research in the Antarctic and the direction of the science itself controlled by the same people.

Most BAS staff agree with Heywood, although some grumble about the unavoid-

able bureaucracy associated with a large institute format. NERC reviews the progress of BAS science annually, and BAS's management every five years or so. In addition, BAS must report twice a year directly to the Department of Education and Science. Producing these reports may divert BAS staff occasionally from their scientific objectives, but is a small price to pay for the protected level of core funding that BAS currently enjoys.

The centralized British approach contrasts strongly with that of most Antarctic nations. In the United States, which supports more Antarctic research than any other nation, there is no directed Antarctic research programme — the dispersed US Antarctic science community relies for its funding on the usual National Science Foundation grant process. Heywood feels that the result is

sometimes a "mixed bag" of research projects.

Given the huge costs and logistic difficulty of transporting people and equipment to Antarctica, Heywood says that BAS's centralized programme represents the most cost effective way of spending the smaller UK budget. "Antarctica isn't a place where you can catch the number 39 bus, do your bit of research, and come home", he says.

But Heywood is aware that some British scientists are hostile to large directed research programmes, arguing that they bleed money from research grant support. Heywood points out that BAS is providing over £1 million over the three years to the end of 1992, to set up and provide logistic support for university research projects in Antarctica.

Peter Aldhous

RESEARCH COMPUTING

Logging-on on the ice

THREE years ago, Grahame Hughes, BAS's computing chief, set himself an ambitious goal: to supply researchers at the British bases in Antarctica with computing facilities as good as those enjoyed by their colleagues in Cambridge.

That aim is close to being realized. The Halley base now has a VAX digital computer with a network of terminals, giving scientists there a smaller version of the network at BAS headquarters. The two networks communicate directly on a daily basis, via the Inmarsat communications satellite. Researchers at Halley can send large data files back to Cambridge to be processed there (or even on supercomputers, via the JANET network), and receive

the results in a matter of days. Before, Halley had only a handful of personal computers, and communicated with Cambridge via facsimile machine.

The new electronic link also means that Hughes and his colleagues can iron out problems that arise in the Halley network from Cambridge, logging on to the Halley machine while data files are transferred.

BAS's new research ship, the *James Clark Ross*, will carry a similar computing network, and Hughes hopes to extend the facility to other British Antarctic bases in the near future. Late last year, he obtained five small secondhand VAX machines at a knock-down price, which are now waiting to be shipped to Antarctica.

P.A.

RESEARCH COLLABORATION

Magnetic poles together

THE British Halley base hosts the Antarctic component of a unique collaboration to study the interactions between the solar wind and the Earth's upper atmosphere, and between the Sun's and the Earth's magnetic fields. Using large radar arrays, British and US physicists are viewing the Antarctic and Arctic skies simultaneously like two "huge television screens", says John Dudeney, head of BAS's Upper Atmospheric Sciences division.

The solar wind is a stream of charged particles, channelled out into the Solar System along the Sun's magnetic field lines. Some of these field lines fuse with those of the Earth, which come to ground at the poles. Charged particles then rain down into the polar atmosphere, causing spectacular aurorae.

PACE (the Polar Anglo-American Conjugate Experiment) relies on two arrays of high-frequency radar antennas. The 250-metre-long Halley array has been built to

complement a similar structure at Goose Bay in Labrador, Canada, run by a team from Johns Hopkins University in Baltimore, Maryland. The radars each scan several million square kilometres of the polar sky for 'ripples' in the ionosphere.

Dudeney hopes that data from PACE, together with satellite observations, will allow physicists to develop a sound quantitative model to predict the behaviour of the ionosphere from observations of the Sun's surface. At the peak of the 11-year solar cycle, as the sunspot number reaches a maximum, strong gusts in the solar wind can cause magnetic storms in the far north and south. In 1989, a particularly severe storm fused a large proportion of Quebec's electric power lines. The disruption could have been minimized had there been some advance warning. With the next solar maximum due at the turn of the century, Dudeney and his colleagues are working to a deadline.

P.A.

Not the 'dirty man'

Cambridge

DAVID Walton has perhaps the most sensitive job at BAS. In addition to heading the Terrestrial and Freshwater Life Sciences Division, he must ensure that the British scientific presence in Antarctica causes the minimum possible damage to the fragile Antarctic environment.

Antarctic environmental protection is a hot political topic, overshadowed by strong feelings about the eventual possibility of oil and minerals exploration. The Antarctic Treaty nations last year met in Vina del Mar, Chile, to begin work on a protocol to protect the Antarctic environment (see *Nature* 348, 269; 29 November 1990 & 348, 570; 13 December 1990). But much work remains to be done, starting at a meeting in Madrid next month. Walton and his staff will have a key role providing scientific support for the British delegation to Madrid.

Walton believes the efforts BAS has made to protect the Antarctic environment have not been given the recognition they deserve. Environmentalist pressure groups have labelled Britain 'the dirty man of Europe', and the assumption is that the tag also applies to the British bases in Antarctica. But "when people turn round to me and say 'you're desecrating the Antarctic', I feel they haven't done their homework", says Walton.

Britain has been monitoring the environmental impact of its bases for longer than any other Antarctic nation. A programme to monitor vegetation changes around the Signy base began in 1964, and the accumulation of heavy metals in lichens has been studied at Rothera since 1976.

Before starting work on a new airstrip at the Rothera base, BAS carried out the first-ever Comprehensive Environmental Evaluation (CEE) of the effects of the development. (The Antarctic Treaty nations decided in 1987 that thorough CEEs should be carried out before any major construction work.) BAS concluded that the airstrip would cause minimal damage outside the small area already affected by the presence of the Rothera base. Environmental monitoring around the airstrip will continue for the next few years, to compare the true environmental impact with BAS's predictions. The results will be published, Walton says.

During the airstrip's construction, which began in January 1990 and is now reaching completion, BAS invited Cassandra Phillips, from the World Wide Fund for Nature, to check that the work was progressing as the CEE had promised. Walton puts a high value on a constructive dialogue with environmentalist groups, but relations occasionally become strained. Greenpeace, for example, is opposed to the construction of new airstrips in Antarctica, so was not placated by the Rothera CEE.

P.A.

A steady expansion

Cambridge

APART from bringing a welcome increase in BAS's annual budget, the resurgence of British Antarctic science sparked by the 1983 Bondi plan (see previous page) has seen an impressive programme of capital investment.

BAS scientists will this June take delivery of the new jewel in their crown, the £35-million research ship *James Clark Ross*, ordered to replace the ageing *John Biscoe*. The *James Clark Ross* will open up a whole range of new research possibilities to BAS's geoscientists and biologists, who will take turns to man the ship in alternate Antarctic summer field seasons.

The geoscientists get the first bite at the cherry, and set sail for Antarctica in September. Peter Barker, head of BAS's Geophysics Division, says that his team has often been forced to rely on research vessels lacking ice-strengthening, such as the *Charles Darwin*.

On board the ice-worthy *James Clark Ross*, BAS geoscientists will be able to sail further south than before, and take 30-metre-deep sediment cores from the sea bed off the Antarctic Peninsula continental shelf. These will provide valuable information about Antarctica's glacial history.

The new ship can also carry a larger supply of compressed air than other British research vessels. This generates the seismic signals used by geophysicists to survey the sea bed. "In the past, we haven't been able to make big enough bangs", says Barker.

Barker hopes that BAS can find a further £1 million to fit the *James Clark Ross* with swath bathymetry equipment, which can produce topographical maps of the sea bed by echo-sounding, surveying across a band twice as wide as the sea depth at a single pass. With an accurate map of the sea bed's topography, BAS scientists would be able to deploy delicate ship-towed instruments, without the risking their loss or damage.

BAS marine biologists are also enthusias-

tic about the new ship. The *James Clark Ross* is equipped with commercial-scale trawling nets, many times larger than those carried by the *John Biscoe*. BAS biologists will use these to sample Antarctic fish and squid populations, to extend their long-term study of the Southern Ocean ecosystem.

The new ship is the most expensive recent addition to Britain's Antarctic research infrastructure, but is only part of a wider programme of improvement. The new crushed rock airstrip at Rothera base should become operational later this year and the southernmost British base, at Halley, has been rebuilt and is now being furnished. BAS is also applying to the government for funds to rebuild the Signy base.

The new Halley base, which should become fully operational during 1992, is a remarkable structure, consisting of one large accommodation block and two smaller blocks housing the laboratories, all built on platforms raised up from the ice. Halley is sited on the floating Brunt Ice Shelf and moves 700 metres away from the Antarctic coast each year. More significantly, snowfall causes the ice surface to rise by about 1 metre a year, engulfing any doorway built at the surface.

The previous four bases at Halley were all built buried in the ice sheet, and people had to enter and leave through shafts to the surface. But a buried base is difficult to repair and maintain. The new Halley platforms can be jacked up manually each year to keep above the rising ice, a feature that will extend their life by five to ten years.

BAS is also spending £5 million to provide a new aircraft to supply its fieldworkers. A secondhand de Havilland Dash-7 is now in Canada, being fitted with the wheel-ski undercarriage that will enable it to land on the ice. The aircraft should come into operation in the 1993-94 field season, with more than twice the range of BAS's four small de Havilland Twin-Otter aircraft.

P.A.



The new Halley base — science on stilts.

Change unsettles Antarctic old hands

Dumont d'Urville

AT Dumont d'Urville, the signs of change — and the need for it — are everywhere. Here, the brand new cohabits with the ageing and the downright obsolete. It is now more than ten years since the founder of Expéditions Polaires Françaises (EPF), the distinguished explorer Paul-Emile Victor, retired. And EPF seems only now ready to disturb the imprint he left. But a continuing cash crisis makes for some odd contradictions at a time of expansion.

A year ago, France decided to boost its polar research with a new Antarctic base and a research institute in Paris (see page 302). "This summer", says Michel Engler, director of EPF and in charge of work on a new landing strip being built at Dumont d'Urville, "there are 13 science programmes, last year there were five and the year before there were two". He expects the number to increase further once the landing strip is finished.

The airstrip construction is a constant reminder that things will never be the same again (which seems against the spirit of the Antarctic Treaty, if not the letter). Perhaps the scar of the five flattened islands will heal in time, but at present the landing strip sears like a welt across the pristine white and blue archipelago.

Meanwhile, a new lease of life is being given to the fleet of traverse vehicles in preparation for the planned new base at Dome C, 1,000 km away. What one veteran calls the "Rolls Royce of the Antarctic" — a brand-new Kässbohrer tractor, complete with stereo cassette player and full heating in the cab — stands next to a handful of 1960s French-made Hotchkiss trucks. Once some of the

most modern vehicles in the Antarctic, they now stand as museum pieces, facing the South Pole. The manufacturer no longer exists and parts cannot be found.

For Michel Pourchet, a chemical engineer from the glaciology laboratory at Grenoble, the construction work on the airstrip, followed by the new base, will mean that facilities at Dumont d'Urville will be neglected. "This year we have no skidoos", he says, "so we will have to use a 40-year-old 'Weasel' that takes three-quarters of an hour to start each morning."

Patrice Godon, head of works at EPF, confesses that it is hard to keep the base up to date. "We are heading for a very tight period", he says.

Overdue new laboratories are being completed, and the dormitory for over-winterers is fairly modern. But the food store and the engineering workshop are converted accommodation from the base erected for the International Geophysical Year (1957–58). They are now so frail that fist-sized holes have appeared. Michel Engler says that, if money is not found to repair the buildings, he will close them down next year.

But he knows there is little hope of replacement in the near future. "You should see the ones at Kerguelen" (one of the French sub-Antarctic islands), says Bernard Morlet, research director of TAAF, and EPF secretary-general. EPF and TAAF have a combined budget deficit of FF20 million this year. While the money for the airstrip and for Dome C is secure, there may be only just enough for a minimal summer research programme at Dumont d'Urville next year.

But not everyone is for modernization and many 'old hands' regret the march of time. There is a definite nostalgia for the old days, when you had to be built like a lumberjack, washed yourself with snow and never complained about the hardships. The five-and-a-half day sea crossing from Hobart, Tasmania, is also part of the sense of adventure. The 65-metre supply-ship *Astrolabe* is



The latest equipment from Austria's ski slopes stands next to the lone surviving Weasel, a contraption of Normandy landing vintage donated to EPF in 1947.

regularly buffeted by 12-metre waves and force-10 gales as it crosses the notorious Southern Ocean twelve times each summer.

This year, *Astrolabe* was stuck in pack-ice for two weeks, 60 km off the coast of Terre Adélie. Last year, gales burst open a rear door, flooding equipment and personal belongings. The ship could have sunk. And, on almost every crossing, about three-quarters of the passengers are seasick for days on end.

"This is the Antarctic, it's not a package tour", say EPF staff, almost proudly. And the long, rough crossing helps to justify an airstrip. This year, before the Gulf war broke out, Prime Minister Michel Rocard was scheduled to visit Terre Adélie.

The present round trip by sea and air would have taken at least three weeks. But, making use of an existing air connection between New Zealand and the US base at McMurdo, on the Ross ice shelf, he could have left Paris mid-week, visited the New Zealand base and been back for a cabinet meeting the following Wednesday. At the end of next year, Terre Adélie will be three days away from Paris.

But even access by sea is becoming more comfortable and tourism is already a reality (despite a cost of \$8,000 for each person). Last year, an estimated 8,000 tourists visited the continent. And, in January, as the *Astrolabe* crashed through the pack-ice after a solitary and gruelling five days at sea, it was a shock to see a manicured liner, the *World Discoverer*, gliding like a swan on a winter lake. But she could not berth at Dumont d'Urville as planned. Unequipped for pack-ice, the liner stood by as the *Astrolabe* ploughed on like an overblown tug. "Thanks for putting on a splendid show", radioed the captain.

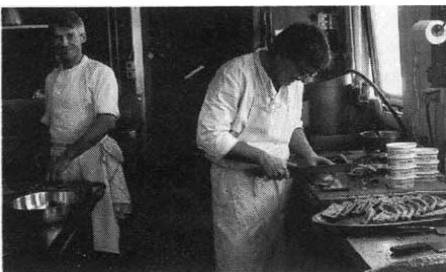
Peter Coles

THE GOOD LIFE

A base marching on its stomach . . .

ON Shackleton's historic 1908 'furthest south' expedition to the Antarctic, after 1,000 miles of slogging and still more than 280 miles from the South Pole, Frank Wild wrote: "We get now less than one-and-a-half cups of pony meat a day, six biscuits . . . three spoonfuls of pemmican; a spoonful of sugar in our tea and cocoa. How is that for more than 50 degrees of frost and a heavy sledge at 14 miles a day and under-clad?" Nowadays, the French do things differently. Gilles Brébant and Christian Simonet, chefs for last year's winterover, have both cooked for President Mitterrand.

In December, direct from Paris aboard the *Astrolabe*, came *quenelles de brochet*, 120 bottles of champagne, 144 bottles of St Emilion Bordeaux, 8,000 snails, hare paté, calves' brains, lobster and 96 kg of frogs' legs. Not a single gram of pemmican or corned beef, though. But, says Simonet, "we do have 25 litres of yellow colouring that we don't know what to do with".



Cuisine is not neglected on the French base: pastry chef Gilles Brébant (left) with chef Christian Simonet.

"Every overwinterer has to put on 3.5 kg, that's the only dietary advice we get", says Brébant. Twice a month, there is a 'grande bouffe' blowout with French wines, hors d'oeuvres, exquisitely presented meat, fish, cheeses and sumptuous patisseries. And this year's pastry chef, 'Tintin' Dumont, gets up every morning at 5:30 to make fresh bread and croissants for breakfast. Eat your heart out Frank!

P.C.

Research takes a back seat

Dumont d'Urville & Paris

"It is complicated", says Françoise Praderie, director of the Earth, Ocean, Space and Environment Department at the French Ministry of Research and Technology (MRT). "I'll do you a drawing." Indeed, French Antarctic research is structured like interconnecting sets of Russian dolls. And even plans for reorganization are unlikely to make life much easier.

For historical reasons, government grants for research in the French Antarctic territory, Terre Adélie and the four sub-Antarctic islands (Kerguelen, Crozet, Amsterdam and St Paul) are managed by Terres Australes et Antarctiques Françaises (TAAF), a government department set up in 1955 specifically to preserve French territorial claims in the areas. TAAF, in turn, is under the tutelage of the Ministry for Overseas Departments and Territories (DOM-TOM).

Last year, TAAF's budget from DOM-TOM comprised about FF45 million out of the civil research budget and a further FF75 million of 'territorial' money. Most of the DOM-TOM money is spent on transport: hire of the Antarctic supply vessel, *Astrolabe* (FF17 million) and running TAAF's own oceanographic ship, the *Marion Dufresne* (FF50 million).

Because the administrative chain of command is so complex, while the unexpected nearly always happens, expeditions to the Antarctic are handled by a separate, non-profit-making association, Expéditions Polaires Françaises (EPF), set up in 1947 by the polar explorer Paul Emile Victor. With grants from TAAF, EPF makes sure everything runs smoothly 'in the field'. It orders clothes, food and fuel, schedules flights, organizes who goes and at which dates and sorts out the chaos when things go wrong.

Since the end of whaling and seal exploitation, the uninhabited TAAF districts have had almost no economic, strategic or political interest. And over the past ten years, DOM-TOM's contribution to TAAF's budget has dwindled from FF103 million to FF75 million.

As a result, EPF — almost entirely dependent on TAAF money — is itself feeling the pinch. Last year it had FF25.5 million (not counting FF20 million for the airstrip being built at Dumont d'Urville). And this year, a FF26-million budget proposal was whittled down to FF20 million by DOM-TOM.

According to Bernard Morlet, secretary general of EPF, this is not enough for both a winter and summer research programme. Fixed costs alone are about FF17.5 million, he says, and during the summer about 190 people will pass through Dumont d'Urville, including 35 incoming and 33 outgoing overwinterers. There is even talk of cancelling next year's programme altogether.

The money crisis has not helped interministerial negotiations to set up a new FF210-million polar research institute, eventually to replace EPF and leaving TAAF something of a clearing house.

In July last year, a change of chief administrator at TAAF brought discussions between the MRT and DOM-TOM to a halt. Nine months later, negotiations have restarted, the major question being the boundary between research and 'minding the shop', as far as TAAF is concerned.

Even when the institute finally gets off the ground, there will be no full-time scientific staff. In France, polar researchers come from CNRS or university laboratories all over the country, while technicians and engineers are either on fixed-term contracts or are 'volontaires à l'aide technique' (VAT) — young men doing their military service — together with a handful of military personnel, such as the surgeon, the radio operator and the helicopter pilots.

If the institute does not simplify funding, it should help France to develop a coherent polar research strategy. At present, there is almost no research in the Arctic and TAAF's scientific committee decides the Antarctic research programmes directly, either on the basis of applications from scientists, or as a result of common orientations decided by the international Scientific Committee on Antarctic Research (SCAR). As a first step, a group of French industrialists has joined

MICROMETEORITES

Prospecting in the ice

Dumont d'Urville

FOR Michel Maurette the Antarctic ice could be a storehouse for extraterrestrial matter going back to the origins of the Solar System — micrometeorites. And to prove he is not on a wild goose chase, Maurette, from the CNRS centre for nuclear and mass spectrometry near Paris, has set out to gather 20,000 micrometeorite particles in under two months. This means melting 300–400 tons of Antarctic ice without contaminating it, thanks to a 'factory' technique developed by Michel Pourchet, a chemical engineer from the University of Grenoble laboratory of glaciology.

Maurette has been working up to this gargantuan task ever since he abandoned Moon dust in 1980. Impressed by the purity of a 1978 Antarctic ice core he was able to study, and its capacity to preserve dust particles, he wanted to test an idea that it would be the perfect place to look for micrometeorites, a much more abundant and possibly more primitive source of extraterrestrial matter than meteorites. About 10,000 tons of micrometeorites measuring 50–100 µm fall to Earth every year, he says, the range of sizes also most abundant in near-Earth space —

Capital philately

ONE of the oddities of the French base at Dumont d'Urville is that it is, technically, the 'capital' of one of France's overseas territories. The overwinter leader is a district administrator, a sort of governor appointed by TAAF. And, as a territory, Terre Adélie can issue its own stamps.

Not surprisingly, this novelty has turned into something of a cottage industry. Last year, postmaster and radio operator Jean-Marie Jaguenaud sold stamps worth a staggering FF310,000. "That's a lot of mail for 33 people", quips one disapproving veteran. And of the 38 mailbags arriving on the second visit of the *Astrolabe*, ten (about 10,000 letters) were from stamp dealers asking for first-day covers and special issues with the Terre Adélie franking mark. Amateur stamp collectors get more personal treatment, a note from the helicopter pilot, or a bit of news.

From 1 January to the time the last ship leaves, says Jaguenaud, most of his time, from 8 in the morning till 10 at night will be spent dealing with post and philately. So, when Marcel 'Big Ben' Renard retires next year, he should have a pretty nest-egg. He has been collecting Terre Adélie first-day covers since the very first base at Port-Martin in the early 1950s. P.C.

with MRT, CNRS, TAAF and EPF to set up 'DIPOL' — a research foundation dedicated to the development of polar engineering technology. P.C.

compared to a "few thousand meteorites over 100 grams". And evidence suggests that over 80 per cent of micrometeorites come from comets, whereas meteorites are fragments of the less primitive asteroids.

In the 1970s pioneering attempts to 'trawl' micrometeorites in the stratosphere came up with a total of 1,000 particles, the biggest measuring 50 µm, explains Maurette. And while a magnetic rake drawn over the Pacific ocean bed in 1976 produced 100,000 magnetic 'spherules', they were all larger than 100 µm. Not only were these bigger than the most abundant micrometeorites — and therefore atypical — but it turned out that they had all melted following heating as they entered Earth's atmosphere.

So, in 1984 Maurette and a team of Danish researchers (led by Claus Hammer of the University of Copenhagen) went to Greenland to test his 'hunch' that ice would be the best preserver of micrometeorites. In Greenland the ice melts naturally, forming blue lakes, with a black sediment, 'cryoconite', on the bottom.

Maurette and Hammer recovered 25 kilograms of cryoconite which proved to be the "best preserved and richest mine of micro-

LANDING STRIP

Engineering feat or blight?

Dumont d'Urville

"REALLY, it is the only solution", says affable Michel Engler with a broad grin, as if he is used to speaking to people who are not convinced. Engler is director of Expéditions Polaires Françaises (EPF) and is in charge of engineering work on the FF100-million landing strip nearing construction at Dumont d'Urville. A feat of engineering that has won reluctant applause from sceptics, the landing strip is to be the springboard for French Antarctic research into the next century.

But construction of the runway conflicts with concerns to protect the environment. As there is no ice-shelf to serve as a temporary runway — as at the US McMurdo base — EPF engineers have had to blow up six islands, using the rubble to form a dyke. And these islands formed the only available nesting site for about 10,000 birds — notably Adélie penguins, Cape pigeons, snow petrels and Wilson's storm petrels.

At present, the only access to Dumont d'Urville is by sea. And until mid-December, a barrier of more than 300 km of ice makes it impossible for the modest class 1 ice-breaker, *Astrolabe*, to reach the base. This restricts the summer research programme to

able than was feared. Greenpeace protestors tried to stop building work in 1988, claiming that the strip cut across the preferred migration path of an important colony of Emperor penguins. Now, says Bretagnolle, two years of observations have shown that the Emperors will either walk around the dike or over it, using specially constructed gentle ramps and encouraged by lifelike dummies. And the 3,000 Adélie penguins reproducing on the destroyed islands have — against expectation — shown that they readily find nests nearby. Bretagnolle estimates that only two or three adult Adélies have been killed since 1984, less than the daily meal of a sea leopard.

More at risk, he says, are the colonies of snow petrel and Cape pigeon. "These birds live for over 30 years. They take about eight years to find a mate and a nest, but keep both for life. If you destroy the nests, you destroy the colony." For these birds, all suitable nest sites in the archipelago were already taken. So Bretagnolle arranged for rocks to be broken on uncolonized islands, to create the right kind of crannies.

Although the emperor penguin colony has been less affected than was feared, says Bretagnolle, he fears that the worst is to

come. "The emperors stampede when frightened", he says. Last May, 7,000 king penguins, mostly chicks, died on the Australian Macquarie Island when the colony stampeded, apparently because of a low-flying aircraft.

Michel Engler says it should not be possible for this to happen at Dumont d'Urville. The emperor colony has already returned to the sea at the height of the summer programme and (in theory) is not in the flight path.

"Now our biggest problem is finding the right plane for the runway", says Engler — a 'cart-before-the-horse' predicament that further fuels critics' accusations of poor planning. And no-one yet knows whether it is possible to predict weather conditions at the base with such accuracy that planes can take off from Hobart on the 6½-hour flight and be certain to be able to land. The biggest risk is from blizzards whipped up by sudden katabatic winds of up to 200 km an hour. So, this year, meteorologists from the national weather centre are installing sensors and building a model of the base for wind-tunnel tests. And there is one final worry, France has a poor ecology image in Australia and there is a slight risk that the powerful Green party in Tasmania might try to ban French flights.

P.C.

meteorites" available at the time. But the smaller grains (less than 100 µm) were inseparable from dust blown by the permanent wind, while 'cocoons' of bacterial material had grown around the larger grains. This made them difficult to study without damage and made analyses of their possible organic content meaningless. The only hope of finding intact micrometeorites was the Antarctic.

In 1988 Maurette and Pourchet set up their equipment in a polar caravan at Cap Prudhomme, 10 km from the Dumont d'Urville base.

Pourchet's 'factory' process involves injecting hot water into a small hole in carefully chosen blue ice (with no fractures), and pumping the water and melted ice back into a boiler. This cycle is repeated, gradually creating a pocket under the surface of the ice, about 1.5 m deep and 2 m long. At the end of the day the melted water is passed through a series of filters to trap the meteorites.

In the two available months of the Antarctic summer Maurette and Pourchet melted 100 tons of ice. And instead of the 500 or so grains they expected, they recovered 5,000 micrometeorites and about the same number of melted cosmic spherules. "About 3,000 of the micrometeorites turned out to be between 50 and 100 µm," says Maurette, the size range most abundant in the Earth's neighbourhood, leading him to believe they might be very well preserved. He attributes the unexpected success of the expedition to a 'superconcentrated layer' of ice between 20 cm and 1.5 m below the surface, where micrometeorites were far more common than at other depths. Analyses of the micrometeorites showed that they were even better preserved than those in Greenland and some still contained friable material.

For Maurette, some of their properties seemed to confirm that they were not fragments of meteorites. And they appeared unlike micrometeorites and interplanetary dust recovered from the stratosphere, suggesting that they may be more primitive. "So," says Maurette, "we have a new puzzle. Are these micrometeorites more primitive than the most primitive meteorites? We don't know. But we have a new population of material, it is very abundant and it is very primitive."

Maurette's current expedition to Antarctica with Michel Pourchet aims to collect new material, making sure "there is absolutely no contamination". Dust and particles of soot from diesel exhaust can look remarkably similar to some extraterrestrial material. But, ironically, the success of this expedition will add to his worries. He already does not know how to meet the increasing demand for micrometeorite samples from different laboratories. Each grain has to be analysed with a scanning electron microscope and then catalogued — a full-time job which he has been doing himself. But a solution seems to be at hand. Last year he helped launch EUROMET (see page 308) and was awarded a grant of FF630,000 over three years for a 'curator' for the collection. P.C.



Dumont d'Urville base (bottom left) and its controversial airstrip. The *Astrolabe* can be seen at its moorings (centre).

the months of January and February, whereas the weather is already good in October. And the feasibility of the proposed Dome C base rests entirely on researchers being able to arrive by air.

Extensive efforts are being made to limit the environmental impact of the airstrip. The TAAF, which has responsibility for the base, has earmarked FF4 million for detailed conservation measures — approved by the environment ministry and by the Scientific Committee on Antarctic Research (SCAR). As part of the arrangement, TAAF pays Vincent Bretagnolle, a biologist from the CNRS Centre for the Study of Wild Animals at Beauvoir-sur-Niort in western France, to supervise the protection measures.

Some problems have proved less intract-

In the bleak midwinter

Dumont d'Urville

"PEOPLE who overwinter have a little bit missing in their brains", jokes Jacques Wiget, a veteran of 26 summer expeditions to Terre Adélie, but who has never stayed for the winter. And, while the 33 overwinterers greet the first supply ship with some enthusiasm — it brings fresh food, news from loved ones and new faces — there is also a tinge of resentment. The influx means the end of a nine-month intimacy which, for some, is so powerful that 'normal' life back home becomes intolerably empty.

At Dumont d'Urville, about one-third of overwinterers are on fixed contracts — the two chefs, the engineers and mechanics — and are paid nearly three times their salary back home. But, says Claude Bachelard, EPF's medical officer, money is rarely the main attraction.

Rather, as 'Paulo' Thiebaud says, it is to "clear off and leave everything". And Patrick, another overwinterer who has been before, feels more at ease on the base than in France. After his second winter his wife left him. And, ever since, he has had neither a permanent address nor a permanent job back in France. He has already applied to overwinter at the new base at Dome C when it is opened.

Nowadays the risks due to the cold or to malnutrition have been almost eliminated. But last winter, one young man suffered severe frostbite when he slipped on the ice and lost his gloves while out walking. He became severely depressed and tried to commit suicide by throwing himself off a cliff.

"Yet", says Bachelard, "the main risks are now psychological", due to poor adaptation to isolation in a small, closed society and to the repetitive, highly automated work.

For most, the experience is a rare and positive part of their lives, giving them more independence and confidence. But there are problems almost every year, despite psychological screening of candidates. "With only two candidates for each post", says Bachelard, "real selection is not possible". A few years ago, a meteorologist walked out into a blizzard to check instruments and never returned. "Was it suicide or an accident? We will never know."

The 'overwintering syndrome' is now well-known, with bouts of aggression, withdrawal and depression. "There are three main risk periods", says Bachelard: March, when the supply ship leaves for the last time, "is when people start to wonder why they came".

Then, in July and August, when it is darkest. "The lack of sunshine works on a man", says Paulo Thiebaud, "it can make him or break him." Finally, there is the moment to return home. This, says Bachelard, is when those who were escaping marital or other difficulties realize that their problems have not gone away.

Traditionally, midwinter (21 June) is a chance to let off steam and is celebrated in Antarctica like Christmas day — with a difference. Last year, celebrations lasted a whole week, including a picnic on the dining-room floor amid cut-out fir-trees, drag acts, rock music and doing just about everything conceivable to one's hair.

Much can depend on the base leader. And when, as last year, it is Claude Chaufrasse, all is practically guaranteed to go smoothly. Chaufrasse has overwintered 11 times. "I get the impression that there are more overwintering problems back in Paris than here", he says. "The essential thing is to respect everyone and not let anyone impose himself", he says. And a potential source of friction is between the different groups or ages of staff.

Most of the laboratory technicians (a third of overwinterers) are *volontaires à l'aide technique* or 'VATs' doing their military service. The remaining third are either civil servants (the meteorologists and the district administrator) or army personnel (the radio operators, the surgeon and one technician).

"Once you have detected the qualities and defects of everyone, you have to try to get the best out of them. It's better to help the chef make his best dish than to let him open a can", says Chaufrasse.

Usually, about 20 per cent of overwinterers come back again. When they have overwintered with Chaufrasse, the figure can be around 50 per cent.

"We are savages, it's shameful", says Paulo Thiebaud when he thinks of his wife left to work in a shop back home. And Dumont d'Urville is not for women — at least not in the winter. During the summer, three or four female researchers, journalists and visitors may pass thought the camp.

But it is unofficial policy not to have mixed winterovers and no one has suggested adopting the German scheme of alternating male and female teams.

For Claude Bachelard, the problem is one of safety — the clinic is not equipped to deal with gynaecological problems and a pregnancy could be very dangerous. For others, women would 'upset things', 'create jealousies' or could not handle the conditions.

Yet women (in photographic form) are present in many laboratories and dormitory rooms, while telephone bills for calls to wives and girlfriends average FF6,000 and can be as high as FF35,000 by the end of the year.

There have been women candidates, says Bachelard, and two passed the psychological tests. But they were later turned down in favour of men. Claude Chaufrasse thinks the French will eventually accept women overwinterers "but not with the present administration, it is too rigid".

Vive la difference? The way is open for any eligible female to test sex discrimination legislation.

P.C.

Dome C: new Antarctic base

Dumont d'Urville

WHEN in 1982, TAAF's scientific council organized a meeting of experts to reflect on French Antarctic research for the 1990s, most agreed that a second base was needed. They also agreed that it should be at Dome C (124° 10' E, 74° 40' S), 1,000 km inland from Dumont d'Urville. Four years after Paul-Emile Victor retired as director, French polar expeditions (EPF) needed a new direction. Without the new base, research at Dumont d'Urville would probably fizzle out, leaving mostly automated collection of weather data.

With Dome C — manned by 15 overwinterers and 25 more researchers in the summer — France hopes to reinforce its good reputation in glaciology, to carry out studies on the ozone layer, in astronomy and in human biology. That the site is in the Australian territory is not a problem. Under the Antarctic Treaty, claims to sovereignty are suspended.

In any event, says research minister Hubert Curien, there is every chance that the base will be international, or at least European.

Dome C has already proved its scientific value. In 1978, Claude Lorius, director of the laboratory of glaciology and environmental geophysics at Grenoble and former president of SCAR, led a successful expedition to the site, with logistical support from the US National Science Foundation and the US Air Force. The team extracted a 950-metre ice core, which is providing palaeoclimatic information on the past 32,000 years.

Now, with growing interest in global climate change, palaeoclimatologists want to compare ice records from the two hemispheres. Dome C should provide the right kind of ice to compare with cores from the Northern Hemisphere such as Eurocore and the Greenland Ice Project.

For Bill Budd, professor of meteorology at Melbourne University, Dome C is "one of the opportunity sites in the Antarctic". The quality of the ice and the existing 950-metre hole make it a good site to attempt to drill the 3.2 km down to the bedrock, he says, surpassing the present 2.2-km core from the Soviet site at Vostock. There is little snowfall at Dome C — so that a relatively long period will be covered by palaeoclimatic studies of a given depth of core, even if seasonal variations may barely be detectable.

At the same time, the ice hardly moves, reducing the risk that a core will contain ice and traces from different sites. At Vostock, the older the ice, the further it has travelled.

According to Gérard Mégie, director of the CNRS aeronomics service and president of the International Ozone Commission, Dome C will also be a key site in the Interna-

tional Network for the Detection of Stratospheric Changes. The network will pool data recorded simultaneously at several proposed sites including the French Alps, Hawaii and New Zealand. At present, France contributes observations using lidars (back-scattering radars) at Dumont d'Urville (in collaboration with the Institute for Research on Electromagnetic Waves, at Florence, Italy), but, being at the edge of the ozone hole, the data obtained are less valuable.

Dome C has two major advantages over existing sites, says Mégie. First, its altitude of 3,200 metres on the Antarctic plateau greatly reduces interference to microwave

instruments caused by water vapour found at coastal stations and up to 1,000 metres above sea level. Second, its latitude makes it "probably the optimum site in the Antarctic", since, especially in the Antarctic spring, there is sufficient daylight for observations that rely on the Sun.

For Mégie, planning for Dome C is "like planning for a space project". "We need an integrated project that takes account of the work environment, the instruments, the scientific objectives." Energy efficiency is one consideration: the new base will incorporate heat conservation and recycling techniques. Current plans are to run the base,

supply aircraft and traverse vehicles on kerosene, so only one kind of fuel will be needed.

The French government has already set aside more than FF36 million over five years (to 1993) for the development of Dome C, including the purchase of new traverse vehicles. But many feel that it will be too costly for France alone. **P.C.**

Peter Coles thanks the TAAF and EPF for their hospitality, with special thanks to Bernard Morlet, Pierre David, Michel Engler and Marcel Renard. He would also like to thank Michel Pourchet, Jacques Wiget and Vincent Bretagnolle for hours of conversation in remote places, and the captain and crew of the Astrolabe for a safe and happy crossing. □

AUSTRALIAN ANTARCTIC TERRITORIES

But when does the real science start?

Mawson & Davis

AFTER eight days at sea with little to relieve the boredom, lassitude and seasickness caused by constant 40-knot winds, it is a relief to sight one's first iceberg. The seas become calm and, with 24-hour daylight, it is quite possible to spend vast periods of time staring at the changing sea ice. For scientists working in the Australian Antarctic Territories, this is probably the last chance for introspection until they return home.

The sea voyage alone would deter any but the most committed researcher. It takes, on average, 12 days to get to Mawson, almost 5,500 km from Hobart, a further 600 km to Davis and over a week's journey to Casey. The round-trip back to Hobart in Tasmania is more than 10,000 km.

Such a timetable is adhered to only when the weather is kind. In the 1986-87 summer, Ian Allison, a principal researcher in glaciology with the Antarctic Division, boarded the *Icebird*, to go to the Bunger Hills on the Shackleton Ice Shelf. The ship arrived at Casey and, unable to get through the thick ice surrounding Shackleton, went on to Mawson and then home. Back in Hobart, Allison then boarded the *Nella Dan* which, after visiting Casey, got stuck in ice for a week in an attempt to get through to the Bunger Hills. Rescued by a Soviet icebreaker, the *Nella Dan* returned home. Allison had spent four months and travelled 20,000 km without once setting foot in his research station.

Such stories are common and contribute to the anxiety on board ship. Scientists going down to the Antarctic for a summer have only six weeks on the continent. The rest of the time is taken up on the voyage. Every day stuck in ice, or delayed because of heavy seas, is a precious day from their limited research time.

Anxieties do not stop once one arrives on land. The stations themselves are similar to mining towns — haphazardly arranged large metal containers make up the bulk of the buildings. Since the late 1970s, all three continental stations have been slowly upgraded.

Very comfortable hotel-like accommodation is available, but is often spurned by the old-timers as being too clinical and protected from the elements. The old 'donga lines' of wooden shacks, some almost 30 years old, let in the snow and wind. Despite their popularity, they are likely to be demolished as a fire hazard. Elsewhere on the base, there are gymnasias, saunas, bars with free alcohol, excellent food cooked by two chefs and the occasional wild party.

The scientific facilities, however, are run down and overcrowded. There is no general science laboratory at Mawson, and at Davis and Casey up to 20 scientists use a laboratory designed for a maximum of five people over the summer. The laboratories are relics of the 1960s — cramped conditions, poorly working fume cupboards and, at Casey, no running water. New laboratories are being built but will not be ready for a couple of years, long after the rest of the station has been completed.

Although the Antarctic Division, the section of the Commonwealth Department of the Environment that controls Antarctic research, provides almost unlimited access to helicopters to ferry scientists on field trips, basic scientific necessities are ignored. There are few standard sets of biological equipment in the laboratories. Scientists are encouraged to bring their own. When I was at Davis, the only spectrophotometer broke down, leaving researchers without data for six weeks. This would have continued over the winter had an experienced scientist not brought her own spectrophotometer on the last ship.

Seasoned scientific visitors know they

must ensure their cargo is loaded and off-loaded at the correct station. Scientific supplies can go to the wrong base and remain there for the entire season.

One of the constant criticisms of the Antarctic Division is that it organizes the running of the stations with little input from the scientists. This lack of recognition is a major source of dissatisfaction among the scientists. They see little evidence of the government's claim that research is the primary reason, under the Antarctic Treaty, for Australia maintaining a presence on the continent. Antarctic research, the scientists complain, has become increasingly devalued and bureaucratized. For instance, on my voyage, scientists were prevented from leaving the ship as it lay off the coast at both Mawson and Davis. While politicians and journalists were ferried off immediately, scientists had to stay behind to help load cargo onto helicopters — often losing up to four valuable days of their tight six-week schedules.

According to Bruce Davis, deputy director of International Antarctic and Southern Ocean Studies at the University of Tasmania, scientists, not bureaucrats, ruled in the Antarctic until the late 1950s. "Once the Antarctic Treaty was signed in 1959, the government realized there were votes in Antarctic research. Before that, the Antarctic had nothing to offer them but nuisance value. It was shunted from portfolio to portfolio. But in the late sixties and seventies, Antarctic science was seen as a way to increase Australia's prestige internationally. All dealings in the Antarctic became politically and bureaucratically rather than science driven. This attitude peaked in 1978 when A\$80 million was allocated towards a massive building programme to increase our physical presence on the continent."

Rex Moncur, director of the Antarctic Division, believes it was the announcement of the rebuilding programme that triggered the disgruntlement of the scientists. "At the time we had a very small shipping capacity of 50 berths, most of which were taken up by construction workers. This meant that, for a



Cosy but cramped — the biology laboratory at Davis.

time, fewer scientists went to the Antarctic. The scientists just didn't see that without this rebuilding programme there would be no science."

In 1984, the Antarctic Division leased a West German supply vessel, the *Icebird*. "With the addition of the *Icebird*, we tripled the number of scientists going to the Antarctic. Now we have a new marine research vessel, the *Aurora Australis*, with the capacity to carry 20 scientists and 20 support staff. Despite this increase in our shipping capacity, there is still this ethos amongst scientists that the rebuilding project is detracting from the science."

Many scientists are unconvinced, waiting

to see whether there is an increased emphasis on research once rebuilding is complete. Moncur believes such an improvement involves long-term issues so it is difficult to see immediate improvements. "We are maintaining a strong presence in the Antarctic through our scientific capacity." Bruce Davis disagrees, and sees no signs yet of improvements in the quality of research. "If we want to have an influence internationally we need to be seen to be doing good science. That means senior scientists working in up-to-date laboratories with appropriate equipment. This and not bloody big red (accommodation) sheds, is the currency of credibility in the Antarctic." **Tania Ewing**

OIL SPILLAGE

Helping the local population

Davis

IN June last year, a petroleum spill of 90,000 litres occurred at the Australian station, Casey. An estimated 40,000 litres remains unrecovered. A further 19,000 litres was spilt there in January this year. Biodegradation of these sites through natural microbial activity is hampered by severe cold and a lack of nutrients. Estimates for the time needed for complete removal of such spills in the Antarctic range up to 600 years, compared with a mere 40 years in the more temperate climate of the Arctic. Research at the neighbouring Davis station, however, has shown that the addition of fertilizer can markedly speed up the breakdown of hydrocarbons by bacteria and fungi.

Since 1988, Elizabeth Kerry of the department of agricultural science at the University of Tasmania, has been conducting small controlled petroleum spills on land near Davis station. Sixteen litres of distillate was deposited in four plots in 1988. This was followed by further spills in 25 plots in the 1989-90 summer, to test the effects of fertilizers and increased water availability on the rate of petroleum breakdown. This summer, Kerry returned to take samples to determine the total content of hydrocarbons and the microbial populations in the soil.

In two years, evaporation has removed 28 per cent of compounds of low molecular weight, up to carbon-12. However, to remove the heavier compounds, help is needed. "To get rid of these heavy compounds needs bacteria and fungi. Most hydrocarbon decomposing microbes work

best at 25 to 37° C. Although isolates (of bacteria and fungi) from Davis have been shown to be active at 0° C, the low temperatures, low water availability and low nutrients all slow down the degradation process", Kerry said.

Initial results from this summer's sampling have shown that the addition of phosphorus and nitrogen to the spills has enhanced degradation almost threefold. Microbial activity in the fertilized soils was also three times that of unfertilized soils.

Spills elsewhere in the world have been 'seeded' with selected hydrocarbon decomposing bacteria (see *Nature* 349, 447; 1991). However, according to Kerry, in more temperate climates such seeding may be unnecessary. "The required range of microorganisms is likely to be present anyway, either having been introduced with the oil or present in the natural environment."

This range of bacteria and fungi is unlikely to be present in the Antarctic. "Antarctica has a more limited microflora and any microorganisms introduced with the oil may not survive the severe climatic conditions."

Part of Kerry's work is to determine if there are enough microorganisms in Antarctic oil spills to degrade a range of petroleum components. But if 'deficiencies' are found, it is unlikely that the introduction of new species of bacteria and fungi would be permitted under Antarctic Treaty restrictions. "New species might become established in natural ecosystems and upset their balance. We would rather learn how to encourage the activity of microbes already present through nutrient enhancement." **T.E.**

GLACIOLOGY

Warming on the shelf

Lambert Glacier, near Mawson

LOCAL warming has caused melt streams in the world's largest glacier to expand by 20 km in the past two years and to create lakes, 10 km in length, where none existed three years ago.

During the 1970s and early 1980s, the International Glaciological Project, involving the Soviet Union, United States, Britain, France, Australia and Japan measured the velocity and dynamics of around 3 million square kilometres of the East Antarctic ice sheet (between 160° and 90° E). But at present, Australia is the only nation conducting long-term systematic studies of the glaciology of the East Antarctic ice sheet.

A similar project for the Lambert Glacier and the Amery Ice Shelf (300 km southeast of Mawson) has been planned by Dr Ian Allison, principal researcher in glaciology at the Antarctic Division, since the mid-1970s. The Lambert-Amery traverse aims to skirt the Lambert Glacier on the 2,500-metre contour line.

This summer, 1,250 km of the traverse was covered. Six men travelled in three turbo-charged tractors. Each tractor, pulling a 70-80 tonne load, represented home to these men for four months.

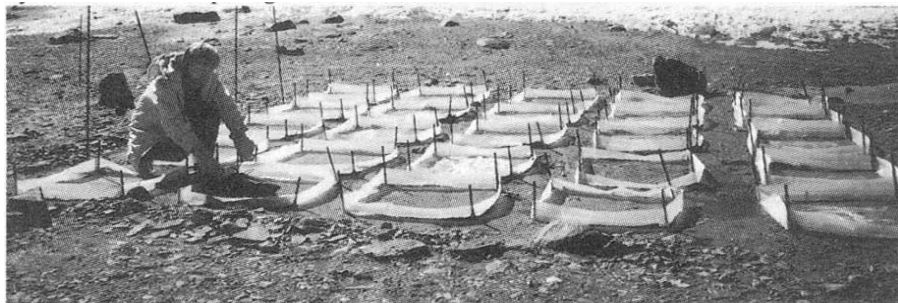
Every 30 km, the party would record the satellite position of a marker placed in the snow. These markers will be re-measured in two years, providing information on the velocity of the glacier. A 100-MHz pulse radar with a 5-kW signal continually recorded the thickness of the ice. Poles placed in the snow every 2 km were measured on the party's return for snow accumulation rates.

In 1992-93 another party, rather than returning to Mawson, will continue to traverse the additional 1,500 km around to Davis.

According to Allison, while the rate at which the Lambert Glacier is moving has changed little, the melt rates are much higher than expected. "The Lambert is moving at a velocity not much different to 22 years ago and the surface elevation hasn't increased. But there is significant surface melt at the seaward end of the glacier at an elevation of 100 metres. Lakes up to 10 km in length have appeared in two years and the melt streams have extended 20 km down the glacier."

Allison believes the increased melt is due to warmer summers in East Antarctica combined with a feedback response. "Snow reflects 80 per cent of sunlight. Melted water reflects only 20 per cent. Sunlight is absorbed and adds to the melting process."

Allison stresses that this glacial melting is not necessarily related to global warming. "This is an indication of local warming over a couple of years. The actual mass balance of the glacier may not have been affected. There may be more snow falling inland which makes up for the melt." **T.E.**



Twenty-five plots of limited oil spillage, with local microbes tended by Elizabeth Kerry.

Penguins keep their distance

Mawson & Hobart

By next summer, Australia's Antarctic stations will be linked into a computer network system allowing year-round automatic data collection. At present, each base is linked to the outside world through four telephone channels. Two are direct-dialling telephone lines; one is a data circuit, operating at 4.8 kilobits, which is linked to the VAX computer network at the Antarctic Division headquarters in Hobart for the storage and retrieval of data, the transfer of meteorological data to the world's weather networks and the transfer of electronic mail; the fourth is a private PABX-PABX tie-line for the transfer of official calls and facsimile messages.

By next summer, this analogue satellite system will be upgraded to digital, according to Peter Magill, engineer-in-charge of telecommunications at the Antarctic Division. "The present four analogue voice channels to each station will be replaced by a single 512 kilobit digital carrier. A first for Antarctica, this will allow the data circuits to be quadrupled to 19.2 kilobits with the remainder used for between 8 and 10 public telephone lines at 32 or 64 kilobits each."

Fibre-optic Ethernet Local Area Networks are to be installed at the stations, creating a network of computers linked by satellite to VAX computers in the Antarctic and in Hobart. Scientists on the Antarctic continent will be able to talk directly to institutions linked into the network. The network will allow transmission of data from field stations all the year round.

WOMEN'S PLACE

Still an embattled minority

Mawson & Davis

Of the four station leaders holding the most senior position in the Australian Antarctic this year, two are women. According to Martin Betts, manager of planning and coordination, the Antarctic Division is making a conscious effort to boost the numbers of women on the Antarctic stations.

While the division denies a policy of positive discrimination, Betts admits that "when we query a person's suitability for a position — if it's a man we are likely to reject him, if it's a woman we are likely to say yes".

Many occupations at the stations, particularly the rebuilding programme, involve few women. However, in a survey of medical staff between 1984 and 1990, 22 per cent were female doctors, the same percentage of women doctors on the Australian Medical Register for this period.

Louise Crossley is one of two women selected for the positions of station leader. The other nine on the short list were men. "My impression is that the Antarctic Division are trying to increase the number of



The penguin weighbridge and custodian.

There will also be some environmental benefits. One of the first projects to be automated will be an Adélie penguin recording system on Bechervaise Island, near Mawson. The system, which records and weighs individual penguins as they move to and from their breeding colony, was developed by a team led by Knowles Kerry, a senior research scientist at the Antarctic Division.

Transponder tags, the size of a domino, will be implanted under the birds' skin. Each tag is powered by batteries that can process and download via VHF radio. Penguins arriving at the beach at Bechervaise Island must cross a detector and weighbridge to reach their colony. "Once this system is working, there will be no need to have researchers lumbering around the colonies weighing penguins and generally interfering in their lives", says Kerry. **T.E.**

women to the point where we represent half of the Antarctic population."

The Australian National Antarctic Research Expeditions (ANARE), the administrative arm of the Antarctic Division, is, according to Crossley, strongly paramilitary and traditionally male but is constrained by public service guidelines. "ANARE is a branch of the public service so the issues of sexual harassment and equal opportunity are, theoretically, covered by legislation. However, there are always sexual overtones. Some men on the station have never worked for a woman and find it hard to accept — particularly in such an isolated situation."

Of the 158 residents at the three continental bases this summer, 19 were female. On her second visit, Anitra Wenden, a doctoral student at Davis, found life easier. "The second time I felt less isolated because there were more women on the station. But the amount of attention on a small number of women is enormous and often the pressure is unbearable. This situation will always exist while there is such a large discrepancy in the sexes." **T.E.**

Long-distance flying

Melbourne

In the summer of 1987, a self-confessed adventurer, Dick Smith, flew a modified light, fixed-wing aircraft to Australia's Antarctic stations, a distance of almost 3,500 km. Apart from being the world's first direct flight between Hobart and the Australian Antarctic Territories, the stunt provided support for those seeking an airlink to the Antarctic.

Of all the nations operating in the Antarctic, Australia has the longest supply lines, at present serviced by two icebreakers. The eight-week round-trip by sea is time-consuming and deters senior scientists from visiting the bases. All three continental stations are predominantly manned by junior scientists, students and volunteers. Many believe a system of intercontinental and intracontinental flights would improve the quality and scope of Australia's Antarctic research.

Opponents of the scheme, however, believe the distances travelled and the weather conditions would make an air support system hazardous and expensive.

In the summer of 1989-90, an attempt was made to build an airstrip at Casey. A freak snowstorm damaged vehicles and halted construction. The project was abandoned (see *Nature* 346,4; 1990). But tests, conducted that winter, simulating landing a 72-tonne Hercules aircraft on the compressed snow runway, were a success.

Casey is the only Australian station at the point of safe return — long-range aircraft can carry enough fuel for the return to Australia if a landing is impossible. Davis and Mawson lie beyond this point.

Professor Bill Budd, from the department of meteorology at the University of Melbourne, has recently prepared a feasibility study on an air service to East Antarctica for the US Cold Regions Research and Engineering Laboratory. Budd believes that with large planes landing at a compressed snow runway at Casey, light fixed-wing aircraft could proceed to Mawson and Davis. "There is an area of solid blue ice 20 km inland from Mawson, which, with a little preparation, could become an airstrip. At Davis we could use the landing strip at the Russian base, Progress, 80 km away, ferrying the passengers back on helicopters."

Budd sees an air system opening up Antarctica to new areas of research and an increase in senior scientists travelling to the continent. "Few senior scientists will take six weeks off for a few days' sampling. With planes we could bring these researchers in, not only in summer but throughout the year, if the weather is right."

But the director of the Antarctic Division, Rex Moncur, is less enthusiastic. "Any airstrip means additional support facilities and

added costs. There is the need to provide facilities and accommodation for stranded passengers — both at the station and at the airstrip — together with the need for improved meteorological forecasting at the actual landing strip. We haven't yet established whether it is possible to overcome all these problems and be completely cost effective."

With Australia's recent A\$10 million investment in the icebreaker *Aurora Australis*, the federal government is unlikely to invest in an expensive and possibly unreliable new transport system.

The Antarctic Division, however, is planning to install automatic year-round weather stations at Casey. "This will give us the long-term data we need about snowfall, solar radiation, melt rates and cloud formation before embarking on another trial. An air system may well cost more than it is worth to get people down there faster. There is certainly a low level of supervision by senior scientists of research at the stations. Because of this we are doing less forefront research. We may be better off spending the money to pay senior scientists to spend up to eight weeks travelling to the continent. This may be cheaper in the end," Moncur says. **T.E.**

How much krill to fish?

Hobart

SCIENTISTS in Australia have a peculiar relationship with krill. In 1985, the world's longest living krill ever held in captivity was inadvertently flushed down a Tasmanian sink. At nine years old, Alan was three times the then average maximum age and was the pride of the Antarctic Division in Hobart.

In the early 1980s, the keeper of Alan, Tom Ikeda, successfully raised individual live krill in glass jars. Today the Antarctic Division in Hobart is home to the world's largest continuous krill-holding facility outside the Antarctic.

Containing between 2,000 and 3,000 krill at a time, the system, developed by Steve Nicol, senior research scientist at the Antarctic Division, is fairly crude, with the krill being kept in large plastic buckets in a cold room. Nicol attributes his success to a clear, continuous water supply, brought in from the east coast of Tasmania.

A planned conversion to a more complex system of several tanks with flow-through water and facilities to monitor gas and food levels may, Nicol fears, end the Antarctic Division's run of good luck. A similar system in Britain has so far been unsuccessful.

Much of the need for krill research arises from two research programmes, both with heavy emphasis on the management of krill fishing. In 1976, the Biological Investigations of Marine Antarctic Systems and Stocks (BIOMASS) was instigated under the auspices of the international Scientific Committee for Antarctic Research (SCAR). The then 12 Antarctic Treaty nations were involved in the BIOMASS project looking at the potential threat to fish stocks by a large krill fishing industry.

In 1982, in response to fears that krill fishing would escalate to the detriment of other fish stocks in the Southern Ocean, the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) was created. The European Communities, the Antarctic Treaty nations and countries fishing in the Southern Ocean agreed to sign. The CCAMLR approach is unique in that it deals with the problems and management of overfishing before it occurs.

But the convention suffers from some important loopholes: it cannot institute any conservation measures unless all members agree to it. Second, any member can escape the requirements of any conservation measures adopted by the convention members by giving notice within 180 days. Nicol is frustrated that, despite krill being the principal reason for the creation of CCAMLR, only the last two annual meetings in Hobart have discussed specific management programmes. "It [krill fishing] is the single biggest economic activity in the Southern Oceans and the treaty, which was raised to prevent overharvesting, has yet to put a resolution to place a cap on the amount of krill that can be fished."

Nicol is frustrated that the Soviet Union and Japan will not agree to a cap on fishing until more is known about krill biology. According to notes from the 1990 CCAMLR meeting, the Soviets, who in 1989-90 harvested 302,376 tonnes, or 80.1 per cent of the season's catch, argue that there is "an abundance of krill" and that talk of management is "premature... and clearly expressed intentions of the fishing nations with regard to their future plans for the krill fishery... should be sufficient to allay the concerns of members".

Certainly little is known about krill biology in its natural state. It is not known why krill swarm, if they are seasonal, why they shrink in size in captivity, and why they regress sexually in response to a lack of food. But the chief concern is that, at 5-10 per cent of the Southern Ocean zooplankton, overfishing of krill could seriously effect the food sources of larger fish, squid, birds and mammals. The most recent CCAMLR meeting held in October last year concluded, according to CCAMLR's executive secretary Dr Darryl Powell, that by the 1991 meeting all CCAMLR signatories must agree to the principle of setting a limit on krill fishing.

Nicol believes this interpretation is overly optimistic. "The Scientific Committee asked 'for an indication of it's best estimates of a precautionary limit for krill in various statistical areas' and 'to identify the various options for the basis on which such a precau-

Dry as dust

Davis

THE harsh, dry winds at the Australian station at Davis are being used to freeze-dry large wooden archaeological artefacts that have spent hundreds of years soaking in water.

According to Wal Ambrose, experimental archaeologist, James Neale, senior technical officer, both from the Research School of Pacific Studies at the Australian National University in Canberra and Ian Godfrey, from the West Australian Maritime Museum, waterlogged archaeological finds are normally treated by replacing the water with a polymer such as polyethylene glycol, which reduces drying and shrinkage and stabilizes the wood. However, for large objects such as the many seventeenth and eighteenth century Dutch sailing ships lying off the West Australian coastline, this technique is too expensive.

Davis station is the driest base in the Antarctic, with little snow and almost constant winds. This means that those living at



Wind assistance: the venturi in action.

Davis have to contend with year-round water restrictions, but conditions are ideal for freeze-drying large wooden objects.

Last summer (1990-91), Ambrose and Neale buried a 3,000-year-old house stump from Papua New Guinea and several pieces of oak from a West Australian shipwreck from the 1840s. Control sections of the stump and shipwreck will be treated using conventional vacuum treatments back in Australia.

The artefacts are lying in a box connected to the outside by a wind tracking funnel-like venturi. "The water-vapour pressure of the ice within the wood is higher than the surrounding air because of suction caused by the venturi. The wind-driven system removes the water vapour from the container, slowly drying the frozen wooden material in the process", Ambrose says. **T.E.**

tionary limit could be established. Furthermore the Commission requested that if an increase in fishing is anticipated (these nations) should notify the Commission within four months of its next meeting'. This is, supposedly, the start of discussions on ways in which the fishery might be managed. It is still a long way from actually implementing management measures." **T.E.**

Concentrating the effort

Melbourne

FEDERAL funding, announced this month (14 March) will bring much of Australia's widely dispersed Antarctic science together in a single research centre in Hobart, Tasmania. In his election speech in March last year, the Prime Minister, Bob Hawke, announced the creation of 50 Cooperative Research Centres (CRCs) by 1994 costing A\$100 million a year.

The centres will combine higher education institutions, the Commonwealth Scientific and Industrial Research Organisation (CSIRO), other Commonwealth and state government research organizations and industry. The aim is to prevent duplication and competition and to encourage cooperation between institutions and organizations conducting research in similar areas. The federal government will provide half of the annual

A\$100 million budget, with the rest provided by the research institutions and the private sector.

The Antarctic and Southern Oceans CRC was one of 15 centres announced for this year. According to Dr Garth Paltridge, director of International Antarctic and Southern Oceans Studies (IASOS) at the University of Tasmania, the centre will be able to provide much-needed coordination for Antarctic research. "Australian Antarctic research is, basically, done by 70 scientists working in 45 separate institutions. Until this centre was announced, we have had no critical mass of Antarctic research outside the 14 scientists based at the Antarctic Division in Hobart."

The new centre will focus on three main areas — the Antarctic and Southern Oceans as a monitor of global climatic change, the

environmental management of Antarctica and the commercial and industrial development of spinoffs from Antarctic research. The centre will also provide postgraduate training in scientific and political aspects of polar research.

The Antarctic and Southern Oceans CRC will be based in Hobart and will incorporate the CSIRO Division of Fisheries, the Bureau of Meteorology, the Bureau of Mineral Resources, the Antarctic Division and the University of Tasmania. The CRC, with an annual budget of A\$9 million, A\$3.5 million of which will be provided by the government, will have its headquarters at IASOS. **T.E.**

Thanks to all at the Australian Antarctic Division, in particular Rex Moncur, Pat Quilty, Steve Nicol, Knowles Kerry, Peter Boyer and Martin Betts. To the captain and crew of the Aurora Australis and to those still in the Antarctic or on their way home — Anitra Wenden, Jim Burgess, Liz Kerry, Ian Allison, Greg Hodge, Andy Brocklesby and Ian Higginbottom. For patience with my questions, my inability to abseil and my sea sickness.

GERMAN RESEARCH

Polarstern – Germany's pride and joy

Bremerhaven

GIVEN a choice, what researcher would take a converted tugboat to the Antarctic when he could take a ride in a luxury liner instead? Research planners in Germany anticipated an enthusiastic response when they decided in the mid-1970s to invest more than DM 200 million (about \$121 million) in *Polarstern* (Pole Star), the most modern polar research ship in the world, and they have not been disappointed. The ship is a big reason why Germany's Antarctic research programme is on the upswing. Gotthilf Hempel, director of the Germany's Alfred Wegener Institute (AWI) and the guiding force behind *Polarstern*, says building a ship that would be attractive to foreign researchers "was the only way Germany could catch up" in polar research. The investment has paid off in research contacts, exchange programmes and, above all, results emerging from the ship's remarkable icebreaking capabilities.

"Everybody is envious of us", says Ger-

hard Dieckmann, a biologist at AWI, not least because *Polarstern* was built with comfort in mind. Researchers sleep at most two to a cabin, and the diversions available (including a swimming pool and sauna), while not posh enough to rival the *Queen Mary*, are far better than those on other polar research ships. Although the trappings might seem a little luxurious for researchers who, after all, are supposed to have their minds on their work, the researchers say that they contribute to higher productivity. "If you didn't feel motivated to spend time aboard ship, it would make it hard to do research", says Wilfried Jokat, a geologist at AWI who spends up to three months every two years aboard *Polarstern*.

Fortunately for the researchers, the quality of the scientific equipment aboard ship matches that of the recreational facilities. Equipped with laboratories for chemical, biological and physical analysis of ice, water, plant and animal samples, *Polarstern* below decks resembles nothing more

than a floating research institute. For example, not only is it possible for researchers to hook up their own computers in their cabins; *Polarstern* also carries two advanced shipboard computers identical to those at AWI. So researchers do not have to wait until returning to Bremerhaven to start analysing their data.

At first, the investment in *Polarstern* paid off mainly in terms of increased foreign contacts. According to Hempel, foreign researchers outnumbered Germans on some of the early cruises. Especially with the United States, Germany established flourishing exchange programmes for young researchers, whose return has begun to improve the quality of the domestic research programme.

But lately, the ship has contributed a growing number of interesting results. In addition to investigating ocean currents as part of a worldwide climate change survey (see next page), *Polarstern* has helped to obtain detailed knowledge about the important ecosystem in and below some of the 20 million square kilometres of sea ice that form each winter above Antarctic waters. And geophysicists such as Jokat are hoping to use data obtained by seismic measurements done from *Polarstern* to develop a better picture of how the ancient land mass called Gondwana evolved into the present jigsaw puzzle of continents, in which all the pieces do not seem to fit together.

In its ten-year lifespan, *Polarstern* has also become a floating laboratory of human relations, with fewer misunderstandings than might have been expected given the many nationalities represented. For instance, Hempel reports that US researchers are "forced to be pushier" than their German counterparts about getting results on every journey because so much of their work de-



depends on "soft" research grants instead of "hard" institutional funding. Nevertheless, says Hempel, people do not really get in each other's way.

National stereotypes have not been borne out aboard *Polarstern*, according to Hempel. "The Finns are sober, and the Italians are among the hardest workers", he says. Furthermore, adds Hempel, the Germans "behaved themselves" more cooperatively as hosts than they would have if they had been among themselves.

One of the strengths of the German polar research programme is interdisciplinary research. Hempel and others at AWI are quick to point out that understanding how creatures can live in the sea ice is intimately related to understanding how ice itself is formed. Every voyage of *Polarstern* leads to several unplanned collaborations among researchers of different disciplines, and often different countries, which Hempel views as a very healthy development.

The opportunity to do interdisciplinary research helps make up for what is seen as the major drawback of *Polarstern*: waiting for others to do their work. Geophysicists and bathymetrists, for example, must make their measurements from a moving ship, whereas biologists and oceanographers usually re-

quire the ship to stand still while they take samples.

Having a number of smaller ships would make it possible for each group to spend more time doing what it came for. But being able to break ice up to one metre thick — and therefore to work in winter — help make *Polarstern* a favourite among German polar researchers, who by now make up three-quarters of the ship's users.

The interdisciplinary approach also characterizes AWI itself, which was founded in 1980 as the newest, and smallest, of Germany's 13 national laboratories (*Grossforschungseinrichtungen*). With a staff of 300 to do research on both poles as well as the ocean, AWI is one of the largest multidisciplinary polar research institutes in the world.

AWI was founded in part to give Germany more of a say in Antarctic affairs. By building a year-round research station, Georg von Neumayer near the Weddell Sea, Germany was able to become a signatory to the Antarctic treaty. AWI is both a logistical and scientific base for the station, which has a crew of nine in winter.

But above all, it is *Polarstern* that has given Germany a greater say in international decisions on the direction of Antarctic research.

Steven Dickman

CLIMATE RESEARCH

Filling in missing pieces

Bremerhaven

As with so much basic research, measuring ocean currents in the Weddell Sea off the Antarctic coast seems deadly dull. But when the project might lead to a better understanding of the world climate and more accurate predictions of global warming, the drudgery is worth the price. By gathering data on the water temperature and salinity, Eberhard Fahrback and his colleagues at the Alfred Wegener Institute will provide one of many components in an ambitious five-year study intended to give researchers a better knowledge of the way the climate is created.

The study, called the World Ocean Circulation experiment (WOCE), is an attempt to fill in several of the missing pieces in the puzzle of climate modelling. Until now, researchers have been working with a very limited set of data. The patterns of ocean movement and ocean-atmosphere exchange in the Southern Ocean are thought to play a key role in the creation of the climate, but because of the lack of shipping traffic and the paucity of scientific expeditions to the region, it is one of the least studied areas in the world.

In addition to its possible role as a sink for carbon dioxide, the region is important because of its role in heat exchange between the ocean and the atmosphere. Sunlight in the tropics warms the ocean water, which flows slowly southward just below the surface until it reaches the Southern Ocean. At the same time, water from the north Atlantic is trans-

ported south at a deeper level. Both water masses give up heat to the atmosphere in the Southern Ocean. The exchange of heat between ocean and atmosphere in the Antarctic is thought to make up a significant part of such heat exchange in the world.

In the Antarctic, a mass of cold, dense water is thus created that sinks to the ocean bottom and begins to flow slowly northwards. As much as 70 per cent of this important water mass, known as Antarctic Bottom Water, is created in the Weddell Sea.

The best available climate models have led researchers to think that this region, representing less than 2 per cent of the overall ocean surface area, is especially sensitive to climate change. But until more data are available, the precise role of the region cannot be determined.

This is where *Polarstern* comes in. By measuring currents in the Weddell Sea, Fahrback and his colleagues hope to provide the data that make better models possible. For example, the figures for the amount of bottom water flowing out of the Weddell Sea vary between 2 million and 9 million cubic metres a second. By measuring the currents over five years, in winter and in summer, Fahrback believes he will be able to improve the accuracy of this figure.

Another bonus will be the eagerly awaited opportunity to compare the modern flow rates with data from earlier geological ages, which have been determined by sampling the Southern Ocean bottom.

S.D.

Meteorites for all

London

IN Antarctic research as in many other fields, the European Communities' (EC) research programme is beginning to make its presence felt. Last September, the EC awarded a 400,000 ECU (about £280,000) three-year grant to the EUROMET project — a pan-European effort to collect and study Antarctic meteorites and cosmic dust.

Antarctica yields a fruitful harvest for meteorite collectors, largely because meteorites are easier to spot on an ice sheet than on stony ground. Antarctic samples also include earlier meteorite falls (see page 300), and are less contaminated with terrestrial material. 'Clean' Antarctic meteorites provide an ideal opportunity to investigate the embryonic Solar System.

The United States and Japan have been collecting Antarctic meteorites for about 15 years. David Drewry, director of the BAS, says the United States and Japan have not kept a "stranglehold" on their collections, but European meteoritists' requests for samples unavoidably take time to process. The EUROMET collection should relieve the pressure, and comes as the Japanese programme is in something of a hiatus, with no field expeditions due for several years.

Ian Franchi, from the Open University (OU), says the first EUROMET field season went well. Blessed by good weather, Franchi and four colleagues collected 264 meteorite fragments in the Frontier Mountains over three weeks straddling the new year. The samples will reach the OU at Milton Keynes in April. Shortly afterwards, a small working group will meet to assess the applications to work on the samples.

The EUROMET team's success in winning EC funding does not mean that 'open season' has been declared for Antarctic scientists to apply for EC money. The project was favoured because of its collaborative nature, involving 40 laboratories in 12 European countries, rather than its Antarctic location. The same goes for a previous major Antarctic project to win EC support: EPOS, a marine ecology expedition on board the German *Polarstern* research ship (see previous page) in the Antarctic summer of 1988–89, provided data for more than 100 scientists from eight EC countries, three other European states and Israel.

But further EC forays into Antarctic science are possible. The EC last year set up the European Committee on Ocean and Polar Science (ECOPS), run jointly with the European Science Foundation (ESF), to consider the potential for future European collaboration. Michele Fratta, from ESF, says ECOPS is now drawing up a proposal for a large European Antarctic ice-core drilling programme, to provide valuable palaeoclimatic data.

Peter Aldhous

A molecular gas streamer feeding the Galactic Centre

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Radio maps of ammonia emission around the nucleus of the Milky Way reveal a long streamer of molecular gas connecting the well known circumnuclear 2-pc ring with molecular clouds 10–20 pc further out. These clouds appear to be interacting with nearby supernova remnants, suggesting that the impact of the supernovae on the gas may have impelled some of it inwards, thus providing a source of fuel for the activity at the Galactic Centre.

THE centre of the Milky Way is the nearest galactic nucleus that we can study. As it is about 100 times nearer than the next galactic system, the Galactic Centre can be studied with high resolution and sensitivity. Here we examine the structure and kinematics of the molecular material around the Galactic Centre. The existence of a circumnuclear structure at the 2-pc scale has been suggested by studies of the dust continuum as well as of spectral lines^{1,2}. In particular, a ringlike structure has been seen in hydrogen cyanide emission which appears to rotate around the nucleus³. Infall from the neutral ring seems to be a good dynamical model for the observed ionized streamers in the immediate vicinity of the Galactic Centre⁴. By modelling the observed velocities as bound motions, a radial mass distribution can be estimated, leading to the suggestion that a black hole may exist at the Galactic Centre^{5,6}. It has been proposed that the neutral ring is an accretion disk which feeds the nucleus³. The question arises: what is feeding the molecular ring or disk itself? The two inner giant molecular clouds, M – 0.02 – 0.07 and M – 0.13 – 0.08, lie adjacent to the central continuum structure, and are excellent candidates for gas reservoirs which may feed the central structures^{7,8}. Our preliminary results hint at a gas streamer connecting the Galactic Centre to the south⁹. Here we show the full morphology of this gas streamer and its sinuous connection to the rest of the ambient molecular material.

Observations

The aperture synthesis maps of the Galactic Centre region were made in the $(J, K) = (3, 3)$ rotational-inversion line of NH_3 using the Very Large Array (VLA) operated by the National Radio Astronomy Observatory. We chose this transition, at an energy of equivalent temperature 100 K above ground, to enhance sensitivity to the highly excited gas in the nuclear region, and to discriminate against material along the line of sight that had a lower excitation energy¹⁰. The sensitivity of the VLA itself is higher than in previous NH_3 studies¹¹. The data were collected during five sessions: 14, 16 and 17 June 1988, and 22 and 23 October 1989. The VLA was in the C/D configuration on these occasions with a maximum projected baseline of 1 km. We used 3C286 for flux calibration and 1733-130 for amplitude and phase calibrations. We synthesized five independent 2' fields. The spectral resolution was 781 kHz (9.8 km s^{-1}) for two of the fields, and 390 kHz (4.9 km s^{-1}) for the remaining three fields. The

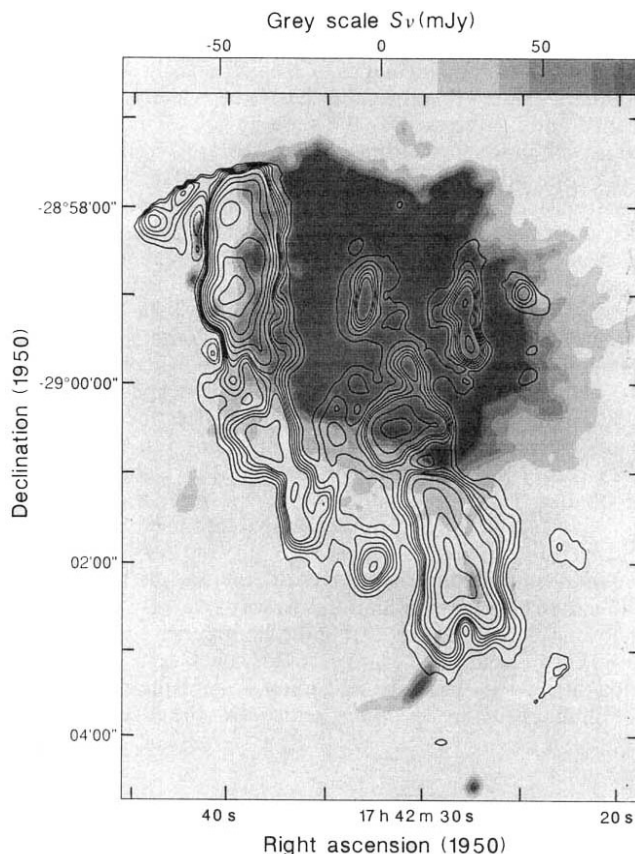


FIG. 1 Integrated NH_3 emission in the $(J, K) = (3, 3)$ line in the Galactic Centre. Five 2' fields have been combined in a mosaic map. To suppress noise at the edge of each synthesized field, primary beam corrections have not been applied but the noise characteristics have been accounted for when averaging overlapping primary beams. The NH_3 emission is shown in contours. The contour levels are: –0.7, 0.7, 1.4, 2.1, 2.8, 3.5, 5.3, 7.0, 8.8, 10.5, 12.3, 14.0, 17.5, 21.0, 24.5, 28.0, 31.5 and $35.0 \text{ Jy km s}^{-1}$ per beam. The synthesized beam is $15'' \times 10''$. For a mean line width of 20 km s^{-1} , the first contour level therefore corresponds to 0.5 K equivalent brightness temperature. The 6-cm continuum emission from the Galactic Centre⁷ is shown in grey scales with the range indicated in Jy. Note the correspondence between the neutral material, as delineated by the NH_3 emission, and the boundaries of the continuum emission.

different fields were stitched together in a linear mosaic, where the individual maps were weighted by the inverse square of the primary beam corrections before being averaged. This takes into account the different noise characteristics at any one position in the map due to the different primary beam attenuations of the synthesized fields. The map shown in Fig. 1 is the integrated $(J, K) = (3, 3)$ intensity map. To suppress noise at the edge of the map, the individual fields were not corrected for primary

beam attenuation in this mosaic. Hence relative intensities are not accurate, but all detected features are real. Maps corrected for the primary beam have been combined and examined; there are no structures that are missing in Fig. 1 although features at the edges of the maps are more intense than represented in the figure. A more detailed analysis of the data will be presented elsewhere. Here we address only the question of the morphology and overall kinematics.

Compressed gas east of Sgr A-East

The integrated intensities of the ammonia emission in Fig. 1 show that the molecular material within 10 pc of the nucleus is closely associated with the continuum emission. This is consistent with the morphology of dust emission at 1 mm wavelength¹². Especially to the east and south, the NH_3 emission closely follows the edge of the Sgr A-East complex and that of a possible second supernova 3' southeast of the nucleus. Close examination of the kinematics of the gas near these continuum boundaries shows that the neutral material is likely to have been pushed and compressed by the supernova.

Figure 2 shows the field east of Sgr A-East. The spectra shown come from specific positions in the molecular cloud. At the emission peak (position 4) hyperfine satellite emission can be seen in the spectrum as a low-level plateau. The satellite line arising from an optically thin cloud in local thermodynamic equilibrium should be about 3% of the main line intensity. The detected satellite intensity therefore indicates the high optical depth of the detected emission. The main emission line profile can be modelled as a single velocity feature, whereas farther to the west and in the continuum complex, the spectra begin to show more complicated structures, best described as double velocity components (for example, positions 5, 6 and 8).

To clarify the kinematics of the region, we show in Fig. 3 declination-velocity diagrams along the right ascension cuts shown in Fig. 2. Towards the central part of the molecular cloud (cut A), the emission is concentrated at $\sim 40 \text{ km s}^{-1}$. Low-level emission wings in this case are due to hyperfine satellite emission rather than high velocity motions. As we move west, the emission at 40 km s^{-1} at the central part of the cloud seems to be displaced to 60 km s^{-1} (cuts C, D and E). This 'backward C' structure, with the velocity shifts being largest toward the centre of the continuum structure and much less towards the edges, is reminis-

cent of the behaviour of an expanding shell. The observed kinematics are consistent with the spherical nature of the supernova: at the edges the radially expanding motions would be directed across the line of sight, whereas closer to the centre expansions would be predominantly along the line of sight. Away from the continuum boundary, the velocities of the neutral gas return to their ambient values. This is consistent with the molecular material being shocked by the supernova at the boundary where it is being stalled. Further evidence of the shock is the presence of CH_3OH and H_2O masers at these boundaries¹³. The velocity shifts ($\sim 20 \text{ km s}^{-1}$) are consistent with those expected for a supernova expanding into a dense molecular cloud. From the observed redshifts of the gas towards the centre of Sgr A-East, and the fact that the observed shell structure in the declination-velocity diagrams does not show a blueshifted counterpart, we suggest that the eastern molecular cloud M-0.02-0.07 must lie behind Sgr A-East if the expansion model is correct. Similar considerations indicate that the supernova to the southeast lies in front of M-0.13-0.08. These kinematical data provide the first direct evidence that the molecular gas lies in the immediate vicinity of Sgr A-East. If Sgr A-East is at the Galactic Centre, then these molecular complexes are indeed as close to the centre as their projected distances on the sky.

Gas streamer near the nucleus

On the integrated intensity map (Fig. 1), in addition to the shell-like features surrounding the continuum structures, the cloud M-0.13-0.08 is clearly seen to the south. Most interestingly, a streamer emanates from the northern edge of this molecular cloud towards the Galactic Centre. It ends at a position in the circumnuclear shell from which NH_3 emission is rather faint. Figure 4 shows an enlargement of the integrated intensities in the central 2' field. The southern streamer is cut off in the south of this picture by the primary beam attenuation. Its continuation can be seen in the mosaic map of Fig. 1. The streamer approaches the centre, but as far as we can tell with the present sensitivity, it does not actually reach the point source known as Sgr A* (RA (1950) = 17 h 42 m 29.335 s, dec. (1950) = $-29^\circ 59' 18.6''$). There are several possible explanations for this observation: (1) the streamer is seen projected against the centre by chance and its termination is unrelated to phenomena at the centre, (2) heating in the centre redistributes the molecular

FIG. 2 *a*, Interpreted NH_3 emission in the field east of the Sgr A-East complex. *b*, Observed spectra at the positions indicated on the integrated intensity map. The velocity resolution is 4.9 km s^{-1} . V_{LSR} is the velocity with respect to the local standard of rest. The vertical lines A-F are the locations of position-velocity diagrams shown in Fig. 3. The low-level emission in the spectra of positions 4 and 9 are not kinematic, they are due to hyperfine satellite emission. Double velocity components seem to be present in a number of the spectra.

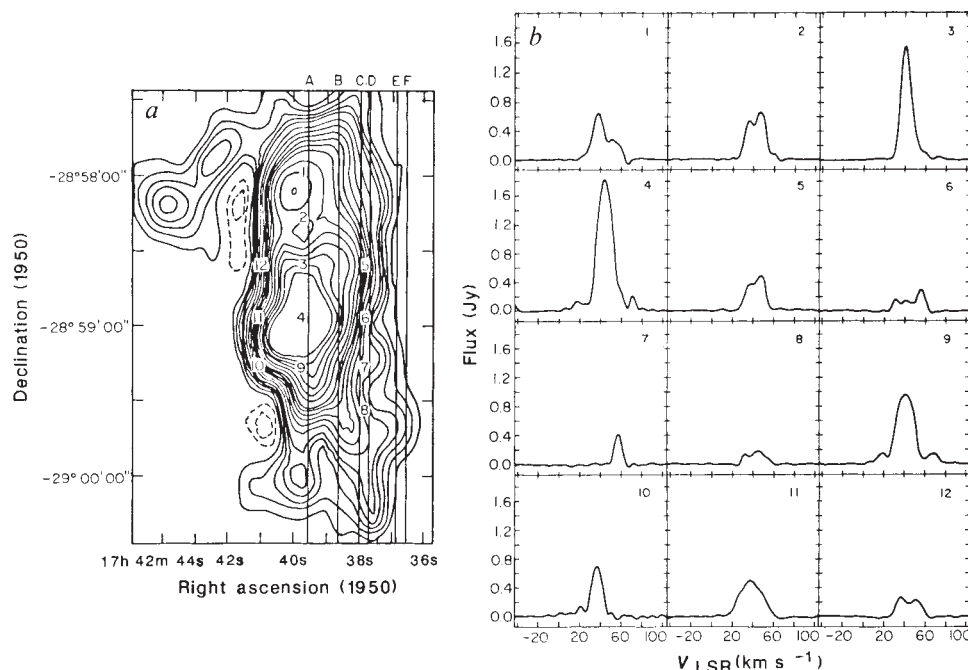
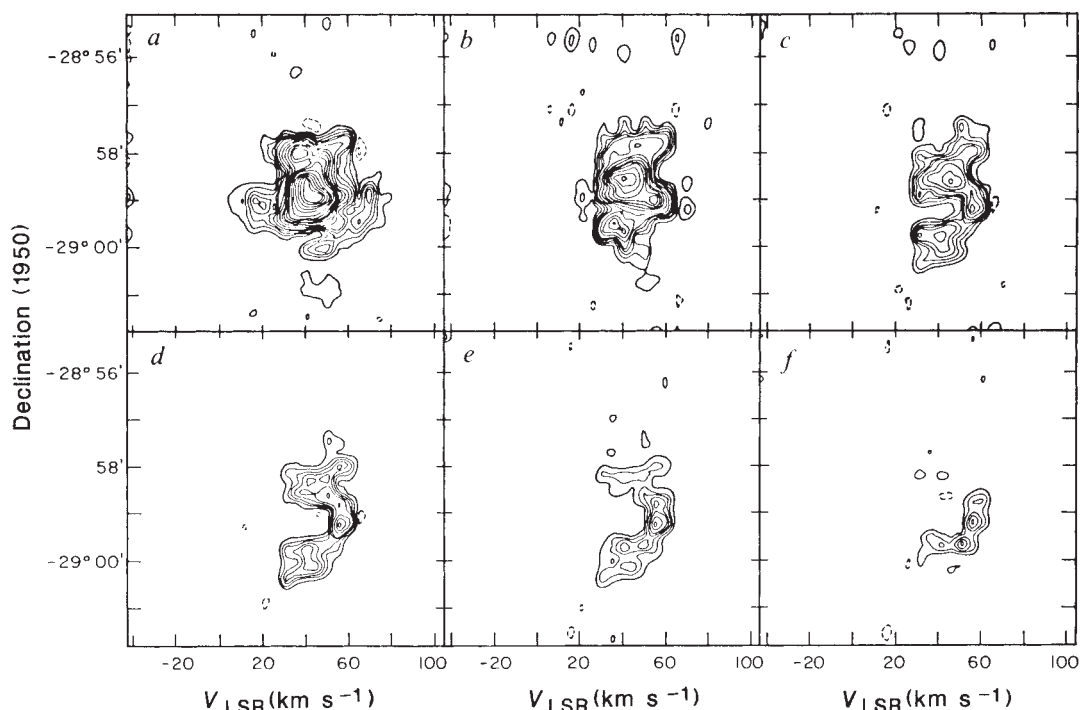


FIG. 3 Declination-velocity diagrams along the directions indicated in Fig. 2. The contour levels are $-0.06, -0.03, 0.03, 0.06, 0.09, 0.12, 0.15, 0.23, 0.30, 0.38, 0.45, 0.53, 0.60, 0.75, 0.90, 1.05, 1.20, 1.35$ and 1.50 Jy per beam. The velocity resolution is 4.9 km s^{-1} . To the west, the emission becomes weaker, and the emission velocity in the central core at 40 km s^{-1} seems to be shifted to 60 km s^{-1} . This can be interpreted as half of a shell-like structure which expands away from us.



population to the higher energy states, thereby weakening the observed transition, and (3) partial conservation of angular momentum results in an increased spin and the settling of the gas into a circumnuclear structure.

We certainly cannot rule out a chance projection of the southern streamer against the Galactic Centre, although all other observed gas features (such as boundaries of supernovae) are well correlated with phenomena at the centre. Comparison with the NH_3 (J, K) = (1, 1) observation both at Nobeyama¹¹ and at the VLA shows that the southern streamer, like the circumnuclear shell, is seen more clearly in the (J, K) = (3, 3) transition. This argues that the gas may be heated because of its proximity to the Galactic Centre. But heating may not be as important as direct ionization effects. There is good agreement between the velocities of the neutral material on the west of the circumnuclear shell and the recombination line velocities of the ionized streamers of Sgr A-West^{14,15}. This supports the idea that material

falls inward from the circumnuclear shell, and that all the material inside is fully ionized. A comparison of 6-, 20- and 90-cm continuum observations shows that the western ionized streamer, seen in absorption against the 90-cm emission, extends further south than was previously thought¹⁶. The spatial correlation between the southern streamer seen in NH_3 and the southern extension of the western ionized streamer is good. This suggests that the southern streamer is indeed closely associated with Sgr A-West.

In our discussions so far, and in the calculations below, we have not assumed the presence of a compact object in the nuclear region, a topic which remains controversial. We merely assume that there is a substantial concentration of mass towards the centre, in the form of stars and possibly other objects, which constitutes the gravitational potential well of our system. We consider the kinematics of the southern streamer with respect to the central gravitational potential. A position-velocity

FIG. 4 Integrated NH_3 emission in the field centred on the Galactic Centre. The NH_3 emission is displayed in contours. The contour levels are $-0.4, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0$ Jy km s^{-1} per beam. The southern streamer is cut off in the south by the primary beam. Its continuation to the south is shown more clearly in Fig. 1. The 1-cm continuum emission constructed from off-line channels is shown as grey scales. The NH_3 emission is closely associated, especially on the western side, with the spiral-like continuum arms which seem to emanate from Sgr A*. This has led to the suggestion that the continuum arms are material which is falling towards the centre from a circumnuclear ring. Note that the southern streamer, which emanates from the southern GMC M-0.13-0.08, ends in the vicinity of the circumnuclear ring without reaching Sgr A*.

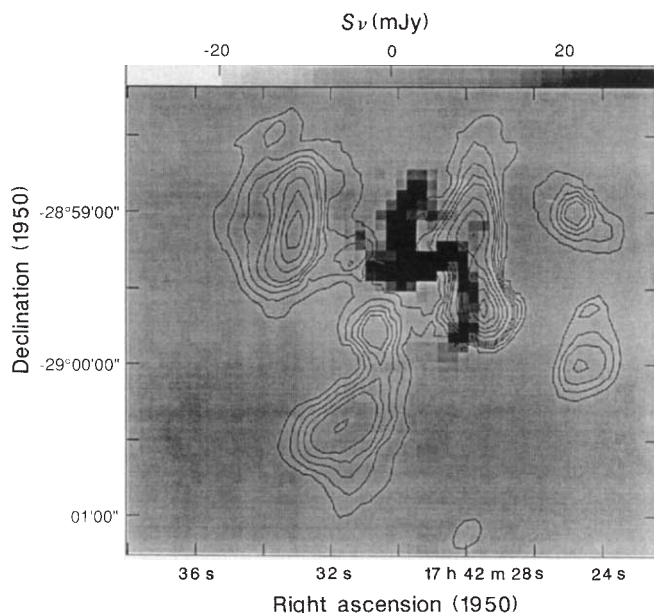


FIG. 5 Position-velocity diagram along the southern streamer. The contour levels are $-0.03, 0.03, 0.06, 0.09, 0.12, 0.15, 0.22, 0.30, 0.37, 0.45, 0.52$ and 0.60 Jy per beam. The velocity resolution is 9.8 km s^{-1} . The mean emission velocity stays fairly constant at 20 km s^{-1} although there are perturbations of $\sim 10 \text{ km s}^{-1}$ along the streamer. The average line width here is $\sim 10 \text{ km s}^{-1}$, a factor of 2 narrower than the emission line width in the molecular complex M -0.02 – 0.07 east of Sgr A-East.

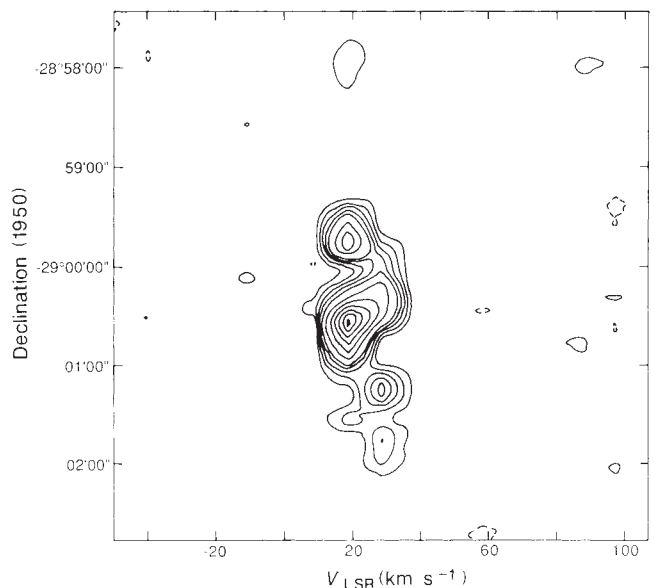


diagram along the southern streamer (Fig. 5) demonstrates that the emission velocity stays fairly constant at 20 km s^{-1} . At first consideration, this seems unreasonable, because the gas streamer should be accelerated as it approaches a central potential. For bound motions, the velocity gradient $(dV/dR) = -0.5 (GM)^{1/2} (R)^{-3/2}$ where V is the velocity, R the radius, M the enclosed mass and G the gravitation constant. For an enclosed mass of $5 \times 10^6 M_{\odot}$, $dV/dR = 73 \text{ km s}^{-1} \text{ pc}^{-1}$ at $R = 1 \text{ pc}$. Similarly by conservation of angular momentum, infalling material at 20 km s^{-1} at 10 pc should have spun up to 100 km s^{-1} at 2 pc . This is of course why gas in the circumnuclear shell at $\sim 45''$ (1.8 pc) has rather large velocities ($\sim 100 \text{ km s}^{-1}$). Because of the steep dependence on R , dV/dR drops to $19 \text{ km s}^{-1} \text{ pc}^{-1}$ at $1'$ from the centre and $6.7 \text{ km s}^{-1} \text{ pc}^{-1}$ at $2'$ from the centre. This is nevertheless a sizeable acceleration which should be detectable. The small value of dV/dR ($< 3 \text{ km s}^{-1} \text{ pc}^{-1}$ along the entire length of the southern streamer) and the narrow line widths ($< 10 \text{ km s}^{-1}$) can be explained in an infall model only if the infall motion is predominantly across the line of sight. This is not inconsistent with the observed morphology of the streamer, which can be interpreted as lying in the plane of the sky. Along the streamer, however, there are large local velocity gradients of the order of $10 \text{ km s}^{-1} \text{ pc}^{-1}$. Whether these gradients and the sinuous morphology of the streamer are due to tidal effects or self gravitation in the streamer is not clear.

If the infall model for the streamer is correct, we can estimate the mass inflow rate, $\dot{M} = 8 \times 10^{-2} M_{\odot} \text{ yr}^{-1}$. We have assumed that $(N_{\text{NH}_3}/N_{\text{H}_2}) = 10^{-7}$ where N is column density, a cross-section of $30''$ (1.2 pc), an infall velocity of 100 km s^{-1} as the material hits the circumnuclear shell, a gas excitation temperature of 100 K , a line width of 10 km s^{-1} and an optical depth of 0.1 for the NH_3 (3, 3) line. Given that the various assumptions, especially the unknown infall velocity, are not rigorous, the estimated inflow rate into the circumnuclear region seems more

than adequate to compensate for the inflow rate directly into the nucleus, which has been estimated to be $\sim 10^{-3}$ to $10^{-2} M_{\odot} \text{ yr}^{-1}$.

Conclusions

From the integrated intensity of ammonia emission, we find that the central circumnuclear region around Sgr A-West seems to be physically connected to the giant molecular clouds farther out by a narrow (1 pc wide) streamer to the south. The detection of this feature is probably due to the sensitivity in this experiment to hot gas at $\sim 100 \text{ K}$. Both kinematics and morphology indicate that the southern streamer originates from M -0.13 – 0.18 . A number of arguments suggest that this streamer reaches the nuclear region: the streamer ends abruptly at the circumnuclear shell, an observation which is consistent with both stabilization because of conservation of angular momentum and ionization in the inner 2 pc ; comparison with NH_3 lines of different excitation indicates that the streamer is hotter than material farther out; and the streamer seems to be well correlated spatially with the extension of the western ionized arm seen in absorption against the 90-cm continuum emission. If the infall model is correct, the absence of a large velocity gradient along the streamer requires that infalling motion is predominantly in the plane of the sky. The infall rate also seems sufficient to replenish the gas in the circumnuclear shell at 2 pc . From the kinematic evidence of the impact of Sgr A-East on M -0.02 – 0.07 , and a second supernova toward the south on M -0.13 – 0.8 , we suggest that such impacts may loosen material from these molecular clouds, which is then captured by the central gravitational-potential. Tidal forces may then stretch the infalling material into a long streamer. If correct, this may be our first opportunity to study the detailed morphology and kinematics of the feeding of molecular material towards the centre of a galaxy. $\square$

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Regulated import and degradation of a cytosolic protein in the yeast vacuole

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The key regulatory enzyme in gluconeogenesis, fructose 1,6-bisphosphatase (FBPase) is subject to glucose-stimulated proteolytic degradation in *Saccharomyces cerevisiae*. This process involves the regulated transfer of FBPase directly from the cytosol into the vacuole or a vacuole-related organelle. Glucose may regulate the production of an FBPase receptor or import factor that is transported to the vacuole through the secretory pathway.

LYSOSOMES are essential for the degradation of macromolecules that are internalized by mammalian cells. Although lysosomal macroautophagocytic uptake of intracellular organelles has long been recognized, it was thought that cytosolic proteins were rapidly proteolysed by cytosolic degradation pathways¹⁻³. Yeast and plant vacuoles are involved in protein degradation and yet, unlike mammalian lysosomes, vacuoles derive their substrates largely from intracellular sources^{4,5}. Global protein degradation during sporulation in yeast requires functional vacuolar proteases⁶, but no selective mechanism for targeting cytosolic proteins to the vacuole has been described.

Vacuolar proteases and catabolite inactivation

Catabolite inhibition of gluconeogenic enzymes could be mediated by vacuolar proteases. Fructose 1,6-bisphosphatase, a regulatory enzyme of the gluconeogenesis pathway, is synthesized when yeast cells are grown in media containing a poor carbon source, but enzyme synthesis is repressed, and existing enzyme is inactivated and degraded when cells are transferred to fresh growth medium that contains glucose^{7,8}. This phenomenon, called catabolite inactivation, could prevent a futile cycle of glucose anabolism and catabolism.

Three independent measurements demonstrated that vacuolar proteases are required *in vivo* for degradation of FBPase. The role of vacuolar proteases was examined in an isogenic pair of strains one of which contained a disrupted *PEP4* gene. *PEP4* encodes a protease that regulates the other vacuolar proteases^{6,9}. Cells transformed with a multicopy plasmid containing the FBPase gene (expression elevated 20–20-fold) were transferred during logarithmic growth from medium containing ethanol as a carbon source (in which FBPase has a half-life of 90 h; ref. 10) to glucose medium (in which FBPase has a half-life of 0.5 h; ref. 10). After 3 h in glucose medium FBPase enzyme activity was reduced by only 50% in *pep4* (mutant cells), but by 90% in *PEP4* cells (Fig. 1a). The 50% decrease could be explained by dilution of existing enzyme during the period of growth in repressing medium, but the 90% could not. Both strains showed no decrease in FBPase activity during continued incubation in ethanol medium. To demonstrate that the influence of *PEP4* on loss of FBPase activity was associated with proteolysis, we examined the level of FBPase polypeptide by immunoblot analysis. Samples similar to those of Fig. 1a were resolved on SDS-PAGE and probed with polyclonal FBPase antiserum (Fig. 1b).

There was glucose-dependent loss of FBPase polypeptide in *PEP4* cells, whereas in *pep4* cells usually about half of the immunoreactive protein was preserved. An unidentified protein with relative molecular mass of about 105,000 that was also detected by the antiserum showed no glucose- or *PEP4*-dependent degradation. Finally, degradation of pre-existing FBPase was monitored by immunoprecipitation of radiolabelled FBPase after chase periods in glucose medium (Fig. 1c). When grown on glucose medium, most of the pre-labelled FBPase was degraded within 2 h in *PEP4* cells, but only about one-half was lost in *pep4* cells. No new FBPase was synthesized by either strain during growth on glucose (not shown). *PEP4*- and glucose-dependent inactivation and degradation were qualitatively similar in cells harbouring only the chromosomal FBPase gene (not shown). Hence, degradation of FBPase by vacuolar proteases is a normal response to glucose and is not merely the consequence of overexpression of FBPase.

Degradation localized to the vacuole

Changes in the location of FBPase induced by growth on glucose were followed using cell fractionation and immunofluorescence microscopy. As cells grew slowly in ethanol medium and were difficult to convert to spheroplasts, an alternative catabolite inactivation regime was developed in which nontransformed cells were grown in an FBPase-inducing medium (1% potassium acetate and 1% glucose) that allowed a more favourable doubling time (~2 h). FBPase inactivation was nearly complete within 1 h after transfer to 2% glucose medium. The kinetics of FBPase inactivation were the same when the non-transformed strains were transferred from ethanol to glucose medium (not shown).

Lysates of *PEP4* and *pep4* cells grown on acetate plus glucose or glucose alone were fractionated on discontinuous Ficoll gradients. Vacuoles were identified by the membrane protein marker dipeptidylaminopeptidase B, and the soluble marker carboxypeptidase Y. To detect the presence of membrane protected FBPase, we exposed cytosol and vacuole-enriched fractions to proteinase K in the presence or absence of Triton X-100 (Tx). Samples were resolved on SDS-PAGE and FBPase detected by immunoblotting. The data in Fig. 2 shows that in wild-type and *pep4* cells, FBPase was in the cytosol, and was degraded by proteinase K (Fig. 2a and c at *t*=0). After 1 h in glucose medium, neither the cytosol nor the vacuole fraction from wild type cells showed detectable FBPase (Fig. 2a and b at *t*=60 min). In contrast, *pep4* lysates displayed FBPase in the vacuole fraction with little in the cytosol. Furthermore, FBPase in the vacuole fraction was insensitive to proteinase K unless detergent was added to lyse the organelle (Fig. 2d, lanes 9–12, without Tx; lanes 13–16, with Tx). Other cytosolic marker proteins such as phosphoglycerate kinase and cytoplasmic invertase fractionated exclusively in the cytosol (data not shown), indicating that FBPase association with the vacuole fraction was selective.

Immunofluorescence microscopy was used to corroborate the dual localization of FBPase. Wild-type and *pep4* cells were fixed and treated with affinity-purified FBPase antibodies at 0, 15, 30, 45 and 60 min after transfer to glucose medium. FITC-conjugated secondary antibody was used to localize FBPase. Cells

were photographed with Nomarski and fluorescence optics, examples of which are shown in Fig. 3. As in the fractionation experiment, FBPase was localized to the cytosol and excluded from the large central vacuoles (Fig. 3a and e). In wild-type cells transferred to glucose medium, FBPase disappeared, but in *pep4* cells the signal gradually redistributed. After 15 min the fluorescence collected in spots that were no longer excluded from the vacuole (Fig. 3f). At 30 min, bright clusters of fluorescence were seen in areas which with Nomarski optics were seen to contain large vacuoles (Fig. 3g). By 60 min the signal diminished, but persisted in spots associated with the vacuolar region (Fig. 3h). Immunofluorescent localization showed that the constitutive form of invertase remained in the cytosol of *PEP4* and *pep4* cells grown on glucose medium (not shown). The results of cell fractionation and visualization experiments suggest that cells respond to glucose by redistributing FBPase from the cytosol into the vacuole or a vacuole-related compartment.

Targeting depends on the secretory pathway

Most proteins that reside in the vacuole are first assembled in the endoplasmic reticulum (ER) and are then diverted to the

vacuole from the Golgi apparatus¹¹. In contrast to most vacuolar proteins FBPase lacks a hydrophobic N-terminal signal peptide, and although the predicted sequence contains three potential N-glycosylation sites, the protein is not glycosylated¹².

sec Mutant strains were used to test the role of the secretory pathway in targeting FBPase to the vacuole. *PEP4 sec* mutants defective in protein translocation from the cytosol into the ER (*sec62*; ref. 13), from the ER to the Golgi apparatus (*sec18*; ref. 14), within the Golgi apparatus (*sec7*; ref. 15), and from mature secretory vesicles to the cell surface (*sec1*; ref. 16) were incubated at a permissive temperature (25 °C) or restrictive temperature (37 °C, or 39 °C for the somewhat leaky *sec7* mutant) after transfer to glucose medium. Extracts were prepared for FBPase enzyme assay (Fig. 4A) or immunoblotting (Fig. 4B). Inactivation and degradation occurred in wild-type and *sec* mutant cells at the permissive temperature, but was retarded in *sec62*, *sec18* or *sec7* cells incubated at the restrictive temperature. *sec1* Mutant cells were not defective in degradation at the restrictive temperature.

The pH gradient across the vacuolar membrane is only marginally involved in normal protein transport to the vacuole¹⁷.

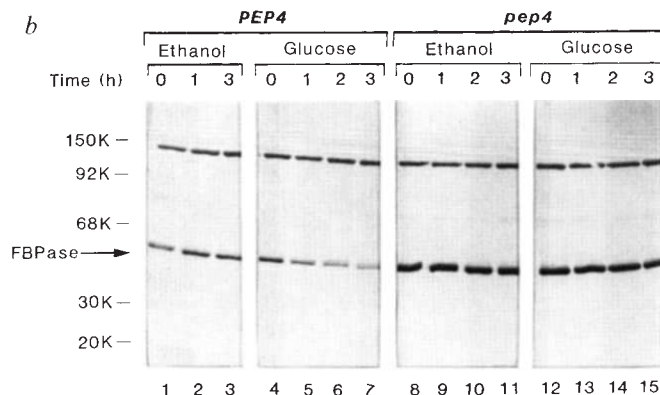
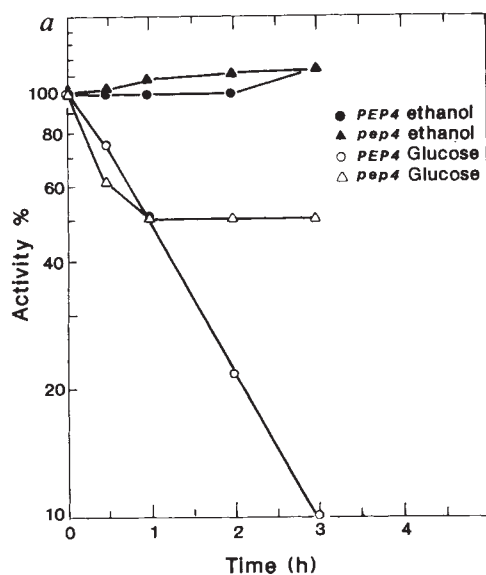
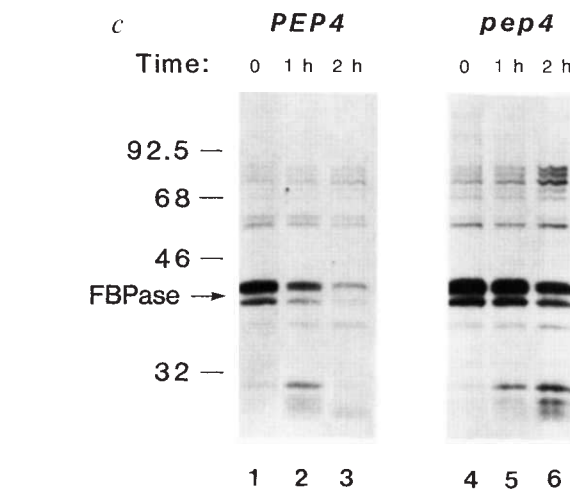


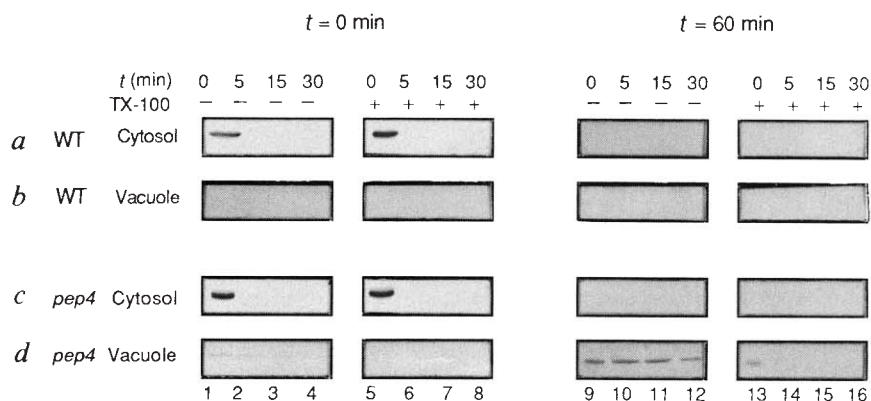
FIG. 1 FBPase is degraded in response to glucose in a *PEP4*-dependent manner. **a**, FBPase activity in *PEP4* and *pep4* cells. *PEP4* was disrupted in strain W303-1B using the integrating plasmid, pTS15 (ref. 28) in which the *TRP1* gene was introduced in place of *URA3*. Wild-type (W303-1B, *Mata*, *ade2*, *leu2-3*, *-112*, *his3*, *trp1*, *ura3*) and *pep4* (W303-BF, *MATa*, *ada2*, *leu2-3*, *-112*, *his3*, *trp1*, *ura3*, *PEP4::TRP*) were transformed by the lithium acetate method²⁹ with a multicopy plasmid containing the FBPase gene³⁰ (D. Fraenkel, Harvard Medical School). Cells were grown in minimal medium containing 2% glucose and supplements (adenine-HCl, uracil, histidine, L-tryptophan, each at 20 $\mu\text{g ml}^{-1}$) to an absorbance at 600 nm (A_{600}) of 10. Cells were diluted to an A_{600} of 1 and transferred to minimal medium containing 2% ethanol, with supplements, for 1 day (A_{600} of 2) to induce FBPase and then shifted to glucose (2%) or ethanol (2%) medium for 0–3 h. Cells ($5A_{600}$) were washed, resuspended in 100 μl of 50 mM HEPES-NaOH, pH 7.2, 5 mM MgSO_4 , 40 mM $(\text{NH}_4)_2\text{SO}_4$, 0.1 mM EDTA and lysed by agitation with glass beads. Unbroken cells were removed and 0.15 mM NADP, 0.2 mM fructose 1,6-bisphosphate, 1 unit each of glucose 6-phosphate isomerase and glucose 6-phosphate dehydrogenase were added. Activity was measured by the formation of NADPH at 340 nm using a Gilford spectrophotometer equipped with a chart recorder. The value was corrected for the fructose 1,6-bisphosphate independent control incubations. **b**, FBPase polypeptide in *PEP4* and *pep4* cells. Cells transformed with the multicopy FBPase gene were treated as described above. Cells ($1A_{600}$ unit ml^{-1}) were obtained at 0, 1, 2 and 3 h after a shift to glucose medium. Cell extracts were electrophoresed on SDS-PAGE and immunoblotted with 1:1,000 dilution of anti-FBPase antiserum obtained from F. Marcus (Chiron Corp., Emeryville, California). Lanes 1–3: *PEP4* in ethanol for 0, 1, and 3 h. Lanes 4–7: *PEP4* in glucose for 0, 1, 2 and 3 h. Lanes 8–11: *pep4* in ethanol for 0–3 h. Lanes



12–15: *pep4* in glucose for 0–3 h. **c**, Radiolabelled FBPase protein in *PEP4* and *pep4* cells. Cells transformed with the multicopy FBPase gene were grown as described above, diluted to $0.5A_{600} \text{ ml}^{-1}$ in minimal medium containing 200 μM ammonium sulphate, 2% ethanol, and leucine, and grown to A_{600} of 3. Cells were labelled with Trans³⁵S-label (30 μCi per A_{600} cells; ICN Radiochemicals) for 3 h and chased in 2% glucose for 0, 1 and 2 h with unlabelled L-met and L-cys (20 $\mu\text{g ml}^{-1}$). Cells were lysed in 200 μl buffer containing 50 mM Tris, 1% SDS, 1 mM PMSF and 10 mM Na_3VO_4 . Immunoprecipitation using anti-FBPase antiserum (5 $\mu\text{l}/A_{600}$ cell equivalent) was performed as described³¹. Lanes 1–3: *PEP4* cells shifted to glucose for 0, 1 and 2 h. Lanes 4–6: *pep4* shifted to glucose for 0, 1 and 2 h.

FIG. 2 FBPase is shifted from cytosol to vacuoles in *pep4* cells. Cytosol and vacuolar rich fractions from wild-type and *pep4* cells were collected following a shift to glucose at $t=0$ and $t=60$ min. Proteinase K was added in the absence (lanes 1–4, lanes 9–12) or presence (lanes 5–8, 13–16) of Triton X-100. *a*, Cytosol from wild-type cells at $t=0$ and $t=60$ min. *b*, Vacuole from wild-type cells at $t=0$ and $t=60$ min. *c*, Cytosol from *pep4* cells at $t=0$ and $t=60$ min. *d*, Vacuole from *pep4* cells at $t=0$ and $t=60$ min.

METHODS. Both wild-type and *pep4* cells were grown to A_{600} of 10–15 in YP (1% Bacto-yeast extract, 2% Bacto-peptone) containing 1% potassium acetate and 1% glucose to induce FBPase activity. Cells ($100 A_{600}$) were collected before and after a shift to 2% glucose medium for 60 min. Spheroplasts ($100 A_{600}$ in 25 ml of 1.2 M sorbitol in YP) were formed by treatment of cells with lyticase (20 units per A_{600} cells)³¹ until the A_{600} of samples diluted 10 fold into water dropped from 0.4 to 0.07. Spheroplasts were resuspended in ice-cold buffer containing 0.2 M sorbitol, 10 mM MES-Tris, pH 6.9 (buffer A) and 12% Ficoll. To achieve an optimal yield of vacuole, DEAE (75 μ l, 0.4 mg ml⁻¹) was added to the spheroplasts and incubated at 4 °C for 1 min, 30 °C for 5 min, and then chilled to 4 °C (J. Shaw and W. Wickner, personal communication). The lysate was placed in an SW41 tube (Beckman, ultraclear) and layered sequentially with 3 ml 8% Ficoll, 4 ml 4% Ficoll and 2 ml 0% Ficoll in buffer A. Samples were centrifuged at 30,000 r.p.m. for 90 min at 4 °C. Cytosol



(1 ml) was collected from the 12% Ficoll layer and a vacuole fraction (1 ml) was collected from the interface between 0 and 4% Ficoll layer. We routinely obtained 20–30% recovery of vacuole (based on the marker protein dipeptidyl aminopeptidase B (ref. 31) using this protocol. Both cytosol and vacuole fractions (200 μ l) were treated with proteinase K (300 μ g ml⁻¹) in the absence and presence of TX-100 (0.4%) for 0, 5, 15, 30 min. Reactions were carried out on ice and terminated by adding samples to 20% TCA. Samples were washed with -20 °C acetone, heated to 95 °C in sample buffer, separated on SDS-PAGE and immunoblotted with anti-FBPase antiserum.

FIG. 3 Immunostaining shows FBPase transferred to vacuole in *pep4* cells following a shift to glucose. Degradation of FBPase was followed in wild-type and *pep4* cells shifted to 2% glucose medium for 0, 30, 45 and 60 min. Cells were visualized by Nomarski optics (left panel). Movement of FBPase was followed by immunofluorescence using affinity-purified anti-FBPase antibodies (right panel). Wild-type cells after shift to glucose at $t=0$ min, *a*; $t=30$ min, *b*; $t=45$ min, *c*; and $t=60$ min, *d*; *pep4* cells after shift to glucose at $t=0$ min, *e*; $t=30$ min, *f*; $t=45$ min, *g*; and $t=60$ min, *h*.

METHODS. Anti-FBPase antiserum (1:50 dilution) was incubated with purified FBPase (10 μ g ml⁻¹) bound to a nitrocellulose strip. FBPase specific antibodies were eluted with 50 mM glycine-HCl, pH 2.2, and dialysed against PBS, pH 7. Antibodies were further purified by incubation with cells carrying a null mutation of the FBPase gene to adsorb nonspecific proteins. FBPase null mutant cells were grown in YP plus 1% potassium acetate and 1% glucose. Cells were fixed with 5% formaldehyde for 1 h at 25 °C and converted to spheroplasts³². Approximately 100 absorbance units of mutant cells were centrifuged and incubated 3 times with antibodies (0.5 ml) for 2 h at 25 °C. Wild-type and *pep4* cells were grown in YP containing 1% potassium acetate and 1% glucose and transferred to YP plus 2% glucose. At 0, 30, 45 and 60 min, 5% formaldehyde was added directly to the culture medium for 1 h at room temperature. Cells were washed and resuspended in 1.2 M sorbitol, 0.1 M KH₂PO₄, pH 7.25, 25 mM β -mercaptoethanol at $5A_{600}$ per ml. Cells were converted to spheroplasts by treatment with zymolase (100 μ g ml⁻¹, 20T, ICN ImmunoBiologicals) for 30 min at 30 °C. Cells were centrifuged and resuspended in 500 μ l of 1.2 M sorbitol, 1% SDS for 30 s. Cells were then centrifuged at 2,600 r.p.m. for 30 s, followed by washing 3–4 times with 1.2 M sorbitol³². Fixed spheroplasts were attached to slides (pretreated with 10 ml of 1% polyethylenimine) for 1 h in a moisture chamber. Cells were blocked with 10 mg ml⁻¹ BSA and 0.05% NP-40 PBS. Purified anti-FBPase antibodies (1:50) were incubated with fixed cells for 2 h at 30 °C. Excess antibodies were washed away and samples treated with FITC-goat-anti-rabbit antibodies (Sigma, 1:500 dilution) for an additional 1 h. Cells were mounted with Citifluor (City University, London), covered with coverglass, and sealed. Cells were visualized by Nomarski phase contrast and immunofluorescence optics using Nikon Optiphot microscope with FX-35 WA camera attached to AFX-11A shutter controller. Photos were taken using Kodak Tmax 3200 and developed.

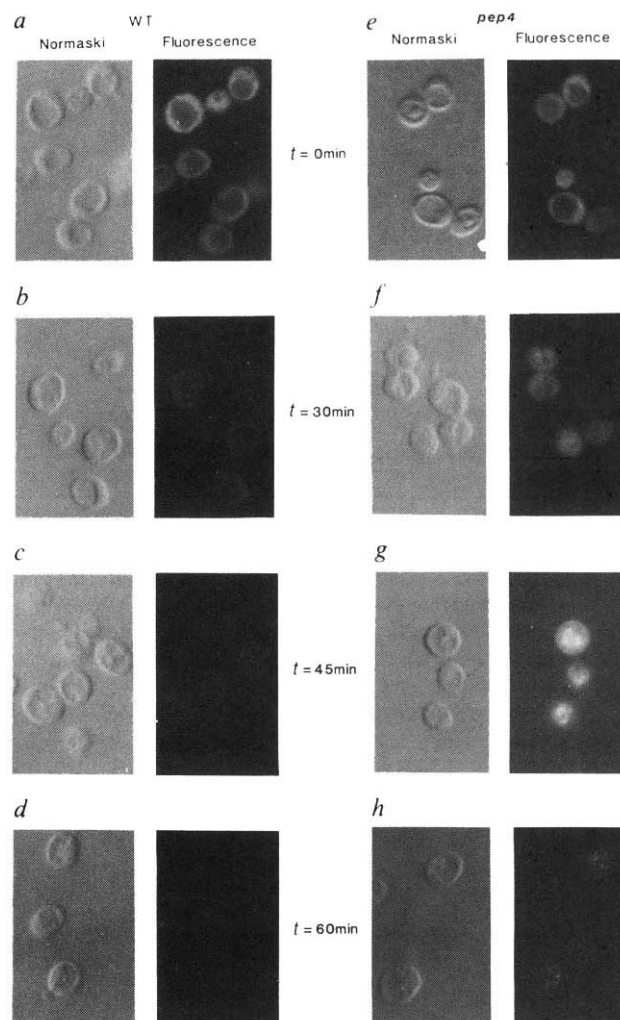
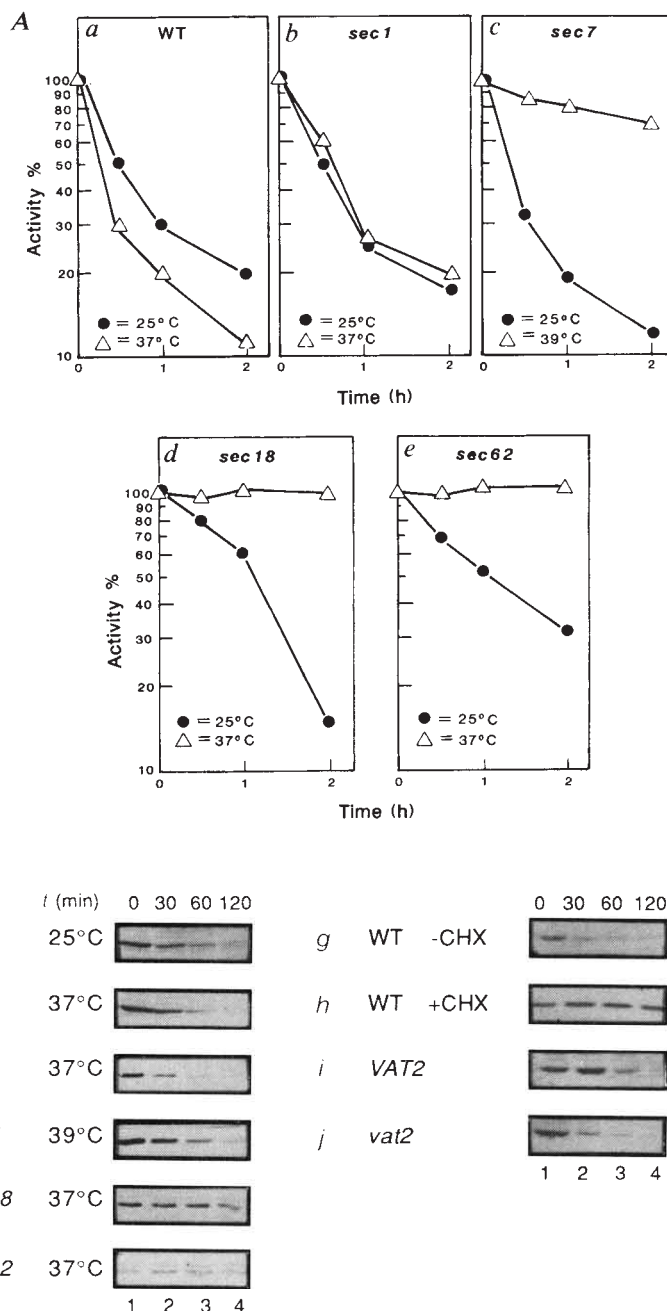


FIG. 4 Degradation of FBPase is delayed in certain secretory mutants. Degradation of FBPase was followed in various *sec* mutant strains incubated at permissive and restrictive temperatures. **A**, Enzyme activity assays; **B**, immunoblot. Glucose was added at either 25 °C or 37 °C (39 °C for *sec7*) for 0, 30, 60, and 120 min. **A**, activity of FBPase at permissive and non-permissive temperature in wild type cells (**a**); *sec1* (**b**); *sec7* (**c**); *sec18* (**d**) and *sec62* (**e**). **B**, FBPase polypeptide in wild-type cells at 25 °C, **a**; wild-type cells at 37 °C, **b**; *sec1* at 37 °C, **c**; *sec7* at 39 °C, **d**; *sec18* at 37 °C, **e**; *sec62* at 37 °C, **f**; wild-type cells at 30 °C without cycloheximide, **g**; wild-type cells at 30 °C with 10 $\mu\text{g ml}^{-1}$ cycloheximide, **h**; *VAT2* cells at 30 °C, **i**; *vat2* at 30 °C, **j**.

METHODS. Wild-type (W303-1B) and *sec-1* (RSY10, *MATa*, *ura3-52*, *leu2-3*, *-112*, *suc2*), *sec7-1* (RSY9, *MATa*, *ura3-52*, *leu2-3*, *-112*, *suc2*), *sec18-1* (RSY11, *MATa*, *ura3-52*, *leu2-3*, *-112*, *suc2*) and *sec62* (RSY529, *MATa*, *ura3-52*, *leu2-3*, *-112*, *his4*) were grown in YP supplemented with 1% potassium acetate and 1% glucose to $A_{600}=10$. Cells were transferred to 2% glucose at a permissive (25 °C) or restrictive temperature (37 °C for most *sec* mutants, 39 °C for *sec7*) for 0, 30, 60, 120 min. Cell extracts were obtained by glass bead lysis and the activity of FBPase was measured as in Fig. 1a. The same samples were also immunoblotted with anti-FBPase antibodies (**B**). Where indicated, cycloheximide (1–0 $\mu\text{g ml}^{-1}$) was added to cells in 2% glucose medium simultaneous to transfer from the acetate/glucose medium. *VAT2* (*MATa*, *leu2-3*, *-112*, *ura3-52*, *ade6*, *gal2*) and *vat2* (*MATa*, *leu2-3*, *-112*, *ura3-52*, *ade6*, *gal2*, *vat2::LEU2*) cells were grown in YP supplemented with 50 mM KH_2PO_4 , 50 mM succinic acid, pH 5, 1% potassium acetate and 1% glucose at 30 °C. Cells were transferred to 2% glucose and degradation of FBPase followed by immunoblotting.



vat2 Mutants that are defective in one of the vacuolar ATPase subunits, and which fail to generate an acidic vacuole, nevertheless contain a nearly full complement of active vacuolar proteases. A pH gradient across the vacuolar membrane was not required for FBPase degradation as the kinetics of this process were normal in *vat2* cells (Fig. 4B, i–j).

Deficient degradation of FBPase in mutants blocked early in the secretory pathway was consistent with either a direct involvement of secretory organelles in the localization of FBPase, or indirectly as carriers of some component involved in importing FBPase into the vacuole. Transfer of cells to glucose medium in the presence of cycloheximide (10 $\mu\text{g ml}^{-1}$) blocked the degradation of FBPase suggesting a requirement for the production of a new transport receptor or vacuolar import protein (Fig. 4B, g–h). The possibility that FBPase was imported into the ER as a prelude to transport to the vacuole was tested by assessing the localization of FBPase in *sec18* cells incubated at 37 °C. If FBPase is imported into the ER before entering the vacuole, then in a *sec18* mutant at the restrictive temperature FBPase

should be in the ER. This possibility was examined using cell fractionation experiments. Lysates prepared from cells before and after transfer to glucose at 37 °C for 90 min contained FBPase that was sensitive to degradation by proteinase K in the absence of detergent (Fig. 5). Thus, as in wild type, the FBPase in *sec18* at the permissive temperature that was not already degraded by vacuolar proteases was in the cytosol. FBPase levels remained constant at the restrictive temperature, indicating that there was no degradation *in vivo*, but unlike the situation in *pep4* cells, the accumulated FBPase remained sensitive to proteinase K in the absence of detergent, which suggests that the enzyme was not sequestered in the ER. Centrifugation of aliquots of the lysate yielded FBPase exclusively in the soluble fractions, consistent with the interpretation that the enzyme remained in the cytosol in *sec18* at 37 °C. As a control we looked at the compartmentation and accessibility of glycosylated α -factor precursor (gpaf), which accumulates within the ER in *sec18* at 37 °C (ref. 13). Approximately half of the gpaf was non-sedimentable, presumably because of some rupture of the

ER during spheroplast lysis. The sedimentable fraction of gp α F was resistant to proteolysis unless detergent was added (Fig. 5c). Thus, FBPase turnover *in vivo*, though dependent on a functioning secretory pathway, is not preceded by import into the ER.

Discussion

Glucose catabolite inactivation of FBPase appears to involve a novel avenue of regulated protein import from the cytosol into the yeast vacuole. FBPase inactivation and degradation are dependent on the function of *PEP4*, the structural gene for vacuolar proteinase A whose activity is required for the maturation of other protease zymogens of the yeast vacuole⁹. Previous conflicting results on the role of these proteases in catabolite inactivation may be attributable to comparison of non-isogenic *PEP4* strains or analysis of cells not maintained in a logarithmic phase of growth^{5,6,18,19}.

The vacuolar import of FBPase requires new protein(s) synthesized in response to glucose. At least part of the requirement for newly synthesized protein may involve the transfer of an assembly factor from the endoplasmic reticulum and Golgi apparatus to the vacuole. *sec* Mutants that are defective early in the secretory pathway block inactivation and degradation of FBPase. The same *sec* mutants block transport of normal

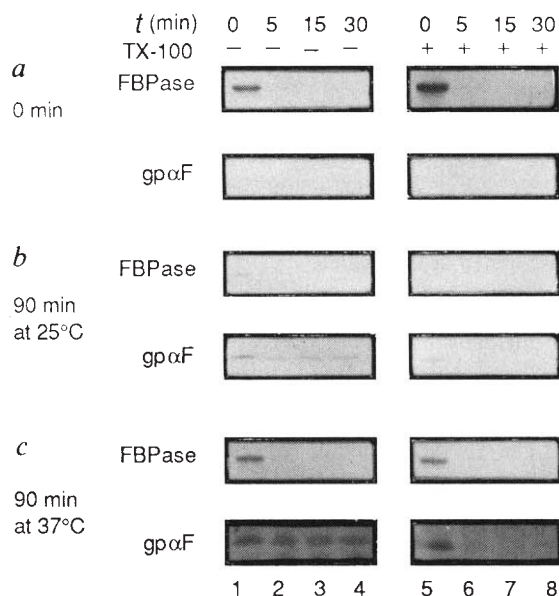


FIG. 5 FBPase persists in the cytosol of *sec18* cells. The *sec18* cells were grown at 25 °C and shifted to glucose at either 25 °C or 37 °C for 90 min. a, *sec18* at *t*=0; b, following a shift to glucose for 90 min at 25 °C; c, following a shift to glucose for 90 min at 37 °C. Proteinase K was added for 0, 5, 15, 30 min in the absence (lanes 1–4) and presence of TX-100 (lanes 5–8). Samples were immunoblotted with anti-FBPase or α factor precursor antibodies.

METHODS. *sec18* cells were grown in YP, 1% potassium acetate and 1% glucose at 25 °C to A_{600} of 10. Cells were transferred to 2% glucose at 25 °C or 37 °C for 90 min. Cells (25 A_{600}) were converted to spheroplasts by lyticase treatment. Cells were centrifuged and resuspended in 1 ml lysis buffer containing 0.3 M sorbitol, 0.1 M KCl, 50 mM Tris, pH 7.5, and 1 mM EDTA. Spheroplasts were transferred to a 2 ml Potter–Elvehjem homogenizer tube on ice and were disrupted by 3 strokes. Extracts were centrifuged at 2000 r.p.m. for 4 min in the HB-4 rotor (DuPont/Sorvall) at 4 °C. The supernatant fraction was further centrifuged at 15,000 r.p.m. for 10 min in a SS34 rotor (DuPont/Sorvall) at 4 °C to sediment intact ER. The resulting pellet was resuspended in 1 ml lysis buffer. Proteinase K (300 mg ml⁻¹) was added in the presence and absence of detergent TX-100 (0.4%). Samples were separated on SDS-PAGE and immunoblotted with anti-FBPase (1:1,000) or anti- α -factor precursor antibodies (1:1,000). gp α F, Core-glycosylated α -factor precursor.

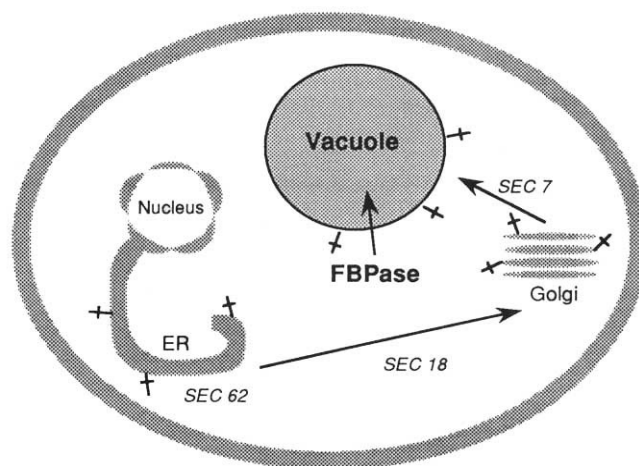


FIG. 6 Pathway of FBPase degradation. FBPase is proposed to translocate directly from the cytosol into the vacuole by a process that is dependent upon a vacuolar membrane protein (+) whose synthesis is induced when cells are shifted to glucose. The new membrane protein traverses early organelles of the secretory pathway en route to the vacuole. Assembly of the new membrane protein requires translocation into the ER (*SEC62*), transport from the ER to the Golgi apparatus (*SEC18*) and transport within the Golgi apparatus (*SEC7*). It is possible that FBPase import occurs into an organelle such as a late Golgi compartment or endosome, rather than into a mature vacuole.

vacuolar precursor proteins by preventing the vesicular transfer of proteins that are first imported into the endoplasmic reticulum¹¹. By contrast, FBPase remains in the cytosol of the *sec18* mutant which accumulates secretory proteins enclosed within the ER. The simplest interpretation of these results is that FBPase import into the vacuole depends on a glucose-induced vacuolar precursor protein that is itself targeted through secretory organelles. Figure 6 depicts such a view, invoking a new vacuolar membrane protein that may specialize in the import of FBPase.

Additional levels of regulation may facilitate FBPase targeting to the vacuole. Phosphorylation of FBPase on Ser 11 by cyclic AMP-dependent protein kinase is increased following incubation of cells in glucose medium²⁰. But phosphorylation and degradation are not obligately coupled. Cyclic AMP supplementation of an auxotrophic strain grown on a poor carbon source produces stable, phosphorylated FBPase²¹. Some other covalent or non-covalent modification could trigger the recruitment of FBPase to the vacuole. We favour the view that FBPase inactivation and degradation occur within the vacuole, rather than inactivation in the cytosol followed by translocation into the vacuole. Inactivation of FBPase does not occur when the enzyme is retained in the cytosol in certain *sec* mutant cells incubated at 37 °C in glucose medium, or wild-type cells treated with cycloheximide.

Two examples of apparently direct import of cytosolic proteins into lysosomes or vacuoles have been reported. A large family of cytosolic proteins that bear a KFERQ localizing signal are degraded in the lysosome when mammalian cells are deprived of serum^{22,23}. This represents a stress response, and consistent with this is the observation that import is stimulated by an hsc70 isozyme^{24–26}. The level at which this response is regulated is not understood. Yeast FBPase contains two sequences with remote KFERQ-like motifs (EKVKQ, QKKLQ; ref. 23), but the role of these domains in catabolite inactivation has not been explored.

Yeast α -mannosidase, a normal component of the inner surface of the vacuolar membrane, is imported post-translationally, apparently directly from the cytosol²⁷. Unlike the import of FBPase, α -mannosidase import is slow, easily saturated by

overproduction of the protein, and not blocked in secretion-defective strains. The two processes could share the same translocation mechanism but utilize different receptor proteins; FBPase import may be greatly facilitated by a glucose-induced receptor.

The mechanism of these translocation events remains to be deciphered. Import of FBPase could be by a unimolecular event, such as is well-known for mitochondrial and secretory protein

translocation. If so, this would require unfolding of the transported molecule which could involve an hsc70 isozyme^{25,26}. Alternatively, protein aggregates clustered on the surface of the vacuole/lysosome could be taken up by a micro- or macroautophagic process, and then degraded along with the internalized membrane. These possibilities may be distinguished by the isolation and characterization of yeast mutants deficient in catabolite inactivation of FBPase. □

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LETTERS TO NATURE

Microwave detection of hydrogen sulphide and methanol in comet Austin (1989c1)

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INFRARED and microwave spectroscopy can be used to identify and study the parent molecules directly sublimed from cometary nuclei. Here we report spectroscopic observations of comet Austin (1989c1) at millimetre wavelengths using the 30-m telescope of the Institut de Radio Astronomie Millimétrique (IRAM). We detected the rotational transitions of hydrogen cyanide and formaldehyde, which were previously observed in comets Halley and Brorsen-Metcalf¹. In addition, we identified hydrogen sulphide and methanol, neither of which has previously been detected in a comet. The presence of hydrogen sulphide provides severe constraints on the formation of cometary nuclei, whereas that of methanol supports the hypothesis that cometary nuclei have retained, at least in part, some primitive material originating from the solar nebula.

The observations reported here were made from 21 to 25 May 1990, when comet Austin was at 1.1 AU from the Sun and at its closest approach to Earth (0.24 AU), with the IRAM 30-m radio telescope at Pico Veleta (Spain). Three receivers could be used simultaneously, allowing us to search for different lines at the same time. The spectrometers were chosen from two banks of 128 × 100-kHz channels and two banks of 512 × 1-MHz

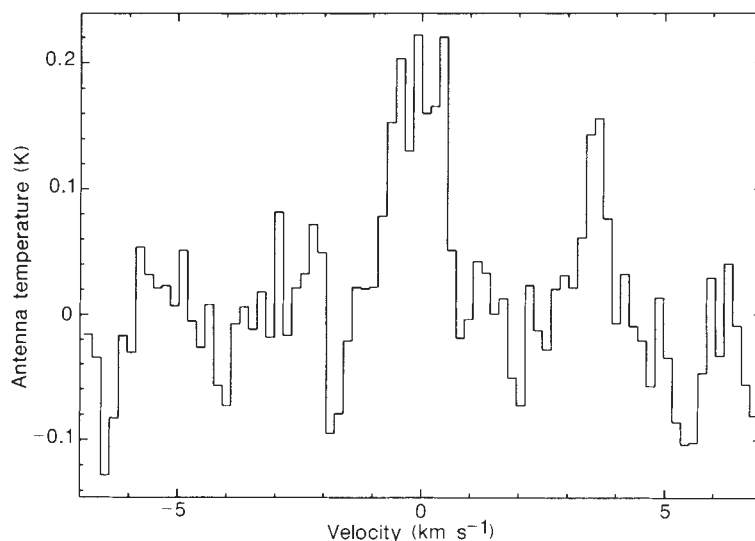
channels. The telescope beam diameter is 14" at 169 GHz. To allow for possible ephemeris uncertainties and tracking errors, and to study the coma extension, we made observations at 6" and 12" north, east, south and west from the nucleus in addition to those aimed at the nucleus.

We detected hydrogen cyanide (HCN) through its J(1–0) and J(3–2) rotational transitions at 89 and 266 GHz, respectively. The formaldehyde (H₂CO) 3₁₂–2₁₁ transition at 226 GHz was observed with a signal-to-noise ratio larger than 10. (A detailed analysis of these results will be presented elsewhere.) Table 1 gives the resulting production rates and abundances relative to water. The HCN production rate is smaller than those we observed previously in comets P/Halley and P/Brorsen-Metcalf (1989)^{2,3}. The H₂CO relative production rate is comparable with that estimated for P/Brorsen-Metcalf³.

To detect hydrogen sulphide (H₂S), we searched for the 1₁₀–1₀₁ transition at 168.762 762 GHz, which is expected to be the most favourable millimetre transition of this molecule because it occurs between low energy levels. We used a double-sideband SIS (superconductor-insulator-superconductor) mixer receiver (the image band being 7.8635 GHz higher); the system temperature was ~1,500 K. The line was unambiguously detected with an area of 0.30 ± 0.04 K km s^{–1} (Fig. 1). The line is centred at the comet nucleus rest velocity, and the line width is 1.4 km s^{–1}, which is typical of all confirmed cometary radio lines. The line was observed on two days with different local oscillator settings, which excludes a spurious detection due to a receiver or spectrometer flaw. Figure 1 is the average of the two days of observation. The weaker and narrower feature at 3.5 km s^{–1} (corresponding to a frequency of 176.6283 GHz in the image band) only appeared in the first day of observation. On the second day, a similar feature appeared at 8.5 km s^{–1} (176.6277 GHz in the image band). Both features may therefore come from the same signal in the image band, but its origin (internal interference or cometary line) is unknown.

Hydrogen sulphide has not previously been identified in a comet, although its presence was suspected from the tentative detection of H₂S⁺ in the visible spectrum of comet IRAS-Araki-Alcock 1983 VII (ref. 4) and from a mass spectrometer signal

FIG. 1 The H_2S $1_{10}-1_{01}$ spectrum of comet Austin (24–25 May 1990). The abscissa scale has been converted to Doppler velocities assuming a line rest frequency of 168.762 762 GHz in the frame of the comet nucleus. Thus the line profile represents the radial velocity distribution of the gas. The frequency resolution is 100 kHz, corresponding to 0.18 km s^{-1} . The intensity scale is main-beam antenna brightness temperature. The telescope beam diameter is $14''$. The spectrum is an average of observations made toward the nucleus and at positions offset by $12''$ north, east, south and west.



at mass 35 attributed to H_3S^+ in the Giotto investigations of comet P/Halley⁵. From the H_2S laboratory ultraviolet absorption spectrum of ref. 6 and the solar reference spectrum of ref. 7, we calculate a lifetime of 4,000 s before photodestruction at 1 AU from the Sun. We assume that this species is expanding from the nucleus with a velocity of 0.8 km s^{-1} (which is in agreement with the observed line width). H_2S is thus confined in the inner coma, and its excitation is governed by collisions. For a preliminary estimate of the production rate, we assume rotational local thermodynamic equilibrium at a temperature⁸ $T_{\text{rot}} = 50 \text{ K}$. This assumption is not critical, because the derived production rate is roughly proportional to the rotational partition function and scales as $T_{\text{rot}}^{1.5}$. The resulting H_2S production rate is $9 \times 10^{25} \text{ s}^{-1}$.

From the cometary water production rate estimated at the time of our observations, we tentatively infer a relative abundance $[\text{H}_2\text{S}]/[\text{H}_2\text{O}]$ of ~ 0.002 . This is an order of magnitude less than the estimated sulphur abundance in cometary volatiles⁹, which is close to the cosmic relative abundance $[\text{S}]/[\text{O}] = 0.02$. The only other sulphur species definitely identified in comets are S_2 and CS (ref. 10), with relative abundances still smaller than that of H_2S . Thus, cometary sulphur might be retained in molecular species so far undetected.

In the analysis of the Giotto mass spectrometer data⁵, an unreasonably large H_2S production rate was obtained when a parent molecule distribution for this molecule was assumed, and a distributed source had to be invoked to interpret the observation. In fact, this problem arose because too short a lifetime for H_2S was used. With the lifetime we have derived, the Giotto data are consistent with an H_2S relative abundance similar to that observed here.

We investigated methanol (CH_3OH) using the same receiver as for H_2S , but with a rejection of 6 dB on the image band; the

system temperature was $\sim 750 \text{ K}$. The spectrometer was a $512 \times 1\text{-MHz}$ bank centred on 145.110 GHz, allowing us to search for several of the $\text{J}(3-2) \Delta K = 0$ transitions simultaneously, but not to resolve the lines (velocity resolution: 2.1 km s^{-1}). We detected two lines with signal-to-noise ratios of 5 and 3 and tentatively detected two other lines at the 2-sigma level (Fig. 2 and Table 1). Observation of other spectral regions with the same spectrometer during this run allowed us to rule out the possibility of faulty channels at these line positions. We also observed the 96 GHz spectral region, where two lines of the $\text{J}(2-1) \Delta K = 0$ transitions are marginally present. Observing several rotational lines of the same species simultaneously is fascinating because it provides clues to its rotational distribution and excitation conditions. In a preliminary calculation, we assumed a unique rotational temperature and obtained the best least-square fit to the data for $T_{\text{rot}} = 22 \text{ K}$. Table 2 gives the relative line intensities expected for $T_{\text{rot}} = 20$ and 70 K . This suggests that methanol is rotationally relaxed, as is observed for other cometary species such as water⁸. It is interesting to note that for this temperature, the other CH_3OH transitions falling in the frequency range of the spectra we observed occur between higher rotational levels and are too weak to be detected. In other words, there are no missing lines in our spectra, and we can have more confidence in our detection. From the CH_3OH laboratory ultraviolet absorption spectrum¹¹ we evaluate a lifetime of $7.7 \times 10^4 \text{ s}$ at 1 AU from the Sun. For $T_{\text{rot}} = 20 \text{ K}$, the resulting CH_3OH production rate is $\sim 5 \times 10^{26} \text{ s}^{-1}$, corresponding to an abundance of 0.01 relative to water.

TABLE 1 Molecular production rates and relative abundances in comet Austin (1989c1)

Molecule	Transition	Production rate (molecules per s^{-1})	Abundance	Ref.
H_2O	OH (ultraviolet)	6.0×10^{28}	1.00	IUE ¹⁸
H_2O	OH (18 cm)	4.0×10^{28}		Nançay ¹⁹
HCN	$\text{J}(1-0)$ 89 GHz	2.5×10^{25}	4×10^{-4}	IRAM
H_2CO	$3_{12}-2_{11}$ 226 GHz	$4 \times 10^{26*}$	6×10^{-3}	IRAM
H_2S	$1_{10}-1_{01}$ 169 GHz	9×10^{25}	2×10^{-3}	IRAM
CH_3OH	145 GHz	5×10^{26}	1×10^{-2}	IRAM

* Assuming a daughter density distribution with a parent scale length of 10^4 km (the production rate would be 10 times smaller if H_2CO were directly sublimated from the nucleus).

TABLE 2 Methanol lines in the 96.7 and 145 GHz spectral regions

Line	Frequency [GHz]	Line area [mK km s^{-1}]	Line ratio		
			Observed	Expected 20 K	Expected 70 K
(2, -1)-(1, -1) E	96.7394	73 ± 38	0.28	0.23	0.22
(2, 0)-(1, 0) A+	96.7414	79 ± 38	0.30	0.40	0.32
(2, 0)-(1, 0) E	96.7446	<105	<0.40	0.21	0.27
(2, 1)-(1, 1) E	96.7555	<105	<0.40	0.11	0.18
(3, 0)-(2, 0) E	145.0937	88 ± 37	0.34	0.53	0.85
(3, -1)-(2, -1) E	145.0975	$143 \pm 52^*$	0.55	0.69	0.84
(3, 0)-(2, 0) A	145.1032	$262 \pm 52^*$	1.00	1.00	1.00
(3, 2)-(2, 2) A-	145.1244	<105	<0.40	0.08	0.32
(3, 2)-(2, 2) E	145.1264	<105	<0.40	0.18	0.40
(3, -2)-(2, -2) E	145.1264			0.15	0.38
(3, 1)-(2, 1) E	145.1319	126 ± 37	0.48	0.31	0.66
(3, 2)-(2, 2) A+	145.1335	<105	<0.40	0.08	0.32

Observed intensities, intensities relative to the (3, 0)-(2, 0) A line and the expected relative intensities at thermal equilibrium are listed.

* Sum of two adjacent channels.

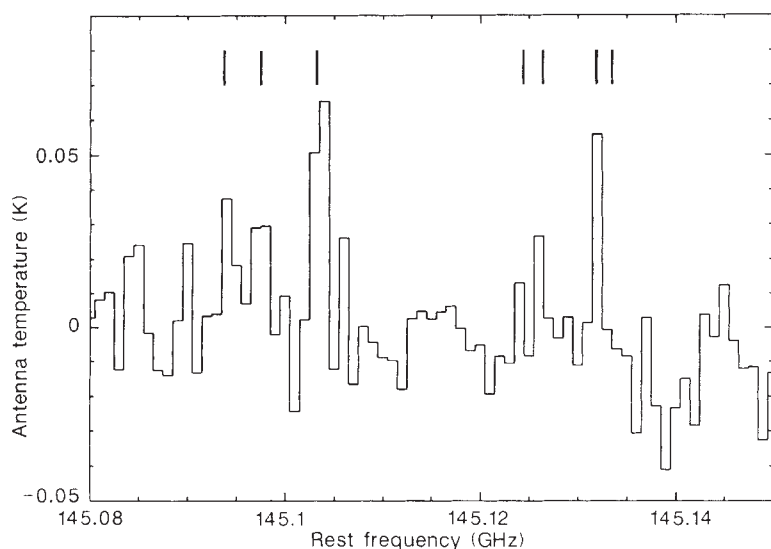
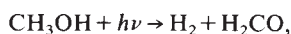


FIG. 2 Transitions of CH_3OH observed around 145 GHz in comet Austin (25 May 1990). The frequency scale is with respect to the frame of the comet nucleus. The resolution is 1 MHz, corresponding to 2.1 km s^{-1} . Tick marks indicate the expected positions of methanol lines (see Table 2). As the resolution is close to the expected line width, the methanol lines may fall in either a single channel, or two adjacent channels. The non-detection of some of the lines is consistent with a low rotational temperature (see text and Table 2). The telescope beam diameter is $16''$. The spectrum is an average of observations made toward the nucleus and at positions offset by $12''$ north, east, south and west.

As one of the main photodissociation channels of methanol is⁷



this molecule may be one of the sources of cometary formaldehyde. Methanol has a strong infrared band at $3.3\text{--}3.5 \mu\text{m}$, and the broad $3.4 \mu\text{m}$ emission band observed in several comets^{12,13} may be due in part to methanol fluorescence. But with an abundance of 0.01, CH_3OH would account for only a small part of the observed infrared band.

Methanol is known to be an abundant constituent of the interstellar medium: its abundance relative to water in the solid phase was recently¹⁴ evaluated as 0.07 in the protostellar source W33A. Methanol cannot survive at high temperatures or in the presence of an ultraviolet field. It has not so far been identified in other solar-system bodies. Therefore, the identification of abundant cometary methanol would provide strong evidence that cometary nuclei retained some of the original matter of the pre-solar nebula. Hydrogen sulphide is not usually an abundant molecule in the interstellar gas, but we should note that it is also present in the solid phase where its abundance is unknown¹⁵. Hydrogen sulphide only condenses at very low temperatures (less than 57 K according to ref. 16). Therefore, its presence in comets would constrain their formation environment.

In addition to the ubiquitous OH radical, four molecular species have now been unambiguously identified in cometary atmospheres by radio spectroscopy. This technique proved to be able to study relatively complex organics such as methanol, or relatively minor species such as HCN or H_2S . It may be anticipated that radio astronomy could achieve an important breakthrough in the near future in the chemical study of comets. This was indeed verified very recently by observations of comet Levy (1990c) which confirmed and extended the present study¹⁷. A more complete analysis of these results will be published elsewhere. □

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Conducting films of C_{60} and C_{70} by alkali-metal doping

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THE recent syntheses^{1,2} of macroscopic quantities of C_{60} have suggested possible applications in host-guest and organic chemistry, tribology, electrochemistry and semiconductor technology. Here we report the preparation of alkali-metal-doped films of C_{60} and C_{70} which have electrical conductivities at room temperature that are comparable to those attained by n-type doped polyacetylene. The highest conductivities observed in the doped films are: 4 S cm^{-1} (Cs/C_{60}), 100 (Rb/C_{60}), 500 (K/C_{60}), 20 (Na/C_{60}), 10 (Li/C_{60}), 2 (K/C_{70}). The doping process is reversed on exposure of the films to the atmosphere. At high doping levels, the films become more resistive. We attribute the conductivity induced in these films to the formation of energy bands from the π orbitals of C_{60} or C_{70} , which become partially filled with carriers on doping. The smaller alkali metal ions should be able to fit into the interstices in the lattice without disrupting the network of contacts between the carbon spheroids. In the case of C_{60} , this would allow the development of an isotropic band structure, and we therefore propose that these materials may constitute the first three-dimensional 'organic' conductors.

Previous highly conducting organic charge-transfer systems have had low-dimensional (quasi-one-dimensional or two-dimensional) electronic structures. In extended systems, the

TABLE 1 Conductivities of alkali-metal-doped films of C_{60} and C_{70}

Film	Dopant	Bath temperature (°C)	Maximum conductivity ($S\text{ cm}^{-1}$)
C_{60}	Li	*	10
C_{60}	Na	180	20
C_{60}	K	130	500
C_{60}	Rb	120	100
C_{60}	Cs	40	4
C_{70}	K	120	2

* Lithium metal in contact with Kovar container and flame-heated.

dimensionality is imposed by the host—for example, polymers (one-dimensional)^{3,4} and graphite (two-dimensional)⁵—whereas in molecular systems the dimensionality is governed by the counter-ion, as in $\kappa\text{-ET}_2\text{Cu}(\text{NCS})_2$ (refs 4, 6), or by crystal packing as in TTF-TCNQ ^{4,6}. The high symmetry and electron affinity⁷⁻¹⁰ of the carbon spheroids (fullerenes)^{1,11} provides a unique opportunity to circumvent these limitations. It should be possible to intercalate small dopant ions into the fullerene crystal without disrupting the network of contacts between the spheroids, thereby generating the first three-dimensional isotropic organic conductor. The structure of C_{60} is comprised of rigid spheres of radius 4.98 Å with face-centred cubic packing ($a = 14.1\text{ Å}$)¹², leaving two vacant tetrahedral sites and one octahedral site per C_{60} molecule of sufficient size to accommodate spheres of radius 1.12 Å and 2.06 Å respectively. The former sites are close to the size of Na^+ and K^+ ions. Furthermore, changes in the packing of the C_{60} lattice could provide a variety of sites for other alkali-metal cations. Occupation of some of these sites and exclusion of extraneous co-intercalants should produce charge transfer from the metals to C_{60} without disrupting the direct contact between C_{60} molecules. Electrochemical experiments^{9,10} have demonstrated that C_{60} undergoes three reversible one-electron reductions, and theoretical considerations indicate that highly reduced states should be accessible under appropriate circumstances⁷. The charge-transfer salt $(\text{Ph}_4\text{P}^+\text{C}_{60}^-) \cdot 2(\text{Ph}_4\text{P}^+\text{Cl}^-)$ has been isolated by electrochemical crystal-growth techniques¹³, but has a conductivity of less than 10^{-5} S cm^{-1} . The low conductivity of this compound is presumably a consequence of the bulky Ph_4P^+ units incorporated in the lattice, which inhibit close contact between the C_{60}^- units. Furthermore, the fullerenes show a marked tendency to crystallize with solvent molecules trapped in the lattice. These considerations, together with the high electron affinity⁷⁻¹⁰ of the fullerenes, led us to attempt to dope thin films of C_{60} and C_{70} with alkali metals. As we wished to obtain highly reduced states of these molecules, we constructed a high-vacuum apparatus which would allow us to measure conductivities *in situ* (Fig. 1).

We deposited thin films of C_{60} and C_{70} on a variety of substrates. The C_{60} and C_{70} samples used as source material in film growth were obtained by reversed-phase column chromatography of fullerite⁴ on octadecylsilanized silica using 40:60 toluene/isopropanol as the eluant. The purities of the C_{60} and C_{70} were checked by high-performance liquid chromatography using ultraviolet detection. High-quality films of C_{60} (C_{70}) of thickness 100–1,000 Å were deposited by high-vacuum sublimation from an alumina crucible regulated at 300 °C (400 °C) at a pressure of 1.5×10^{-6} torr. The C_{60} (C_{70}) films appeared smooth and pale yellow (pink) to the eye and were poorly crystalline as evidenced by X-ray diffraction. Infrared spectra of the C_{60} films deposited on KBr substrates showed the four characteristic absorption peaks of C_{60} (ref. 1) with no evidence of contaminants.

For the conductivity measurements, the films were deposited on glass slides which had been precoated with 1,000-Å-thick stripes or pads of evaporated silver metal. The deposited silver

metal was bonded to silver wires with silver epoxy which were in turn connected to tungsten connectors by platinum wires (Fig. 1). A high-vacuum glass apparatus was constructed with which we could monitor continuously the film conductivity in a variety of electrical configurations. The measured two-probe resistance in the pristine films was greater than $10^{10}\text{ }\Omega$, which, for a film surface area of $1 \times 1\text{ cm}$ and thickness 400 Å, implied a conductivity of less than 10^{-5} S cm^{-1} . Here we describe conductivities below this value as insulating.

The conductivity apparatus was loaded with the alkali-metal dopant in a dry box, before evacuation to a pressure at the pump of about 10^{-5} torr. Initial experiments were conducted with a two-probe apparatus, and higher conductivities confirmed in a four-probe van der Pauw configuration. The bottom of the apparatus was immersed in an oil bath so that the oil level was 5–8 cm below the film, and the temperature raised slowly until conductivity in the film could be detected. All of the doping experiments showed qualitatively the same behaviour—the conductivity first increased by several orders of magnitude and then decreased, usually to a point below our threshold of detection. Using caesium as dopant and with the heating bath at 40 °C, we detected conductivity in a C_{60} film after 2 h. On continued heating, the conductivity increased to a maximum value of 4 S cm^{-1} over an additional period of 2 h before decreasing to a final value of 0.05 S cm^{-1} over a further period of about 4 h. At this point the film had darkened and the initially smooth surface was distinctly granular and rough. The disruption of the C_{60} film is presumably a reflection of the difficulty in incorporating the large Cs^+ counter-ion (radius 1.67 Å) in the lattice. Doping of the C_{60} films with the other alkali metals occurred on similar timescales and led to magenta films with fairly good surface quality, which remained specular. The C_{70} film showed little colour change on doping. The results for the combinations tested are summarized in Table 1.

Of the C_{60} dopants, potassium gave rise to the highest conductivities, and we therefore selected this system for further study.

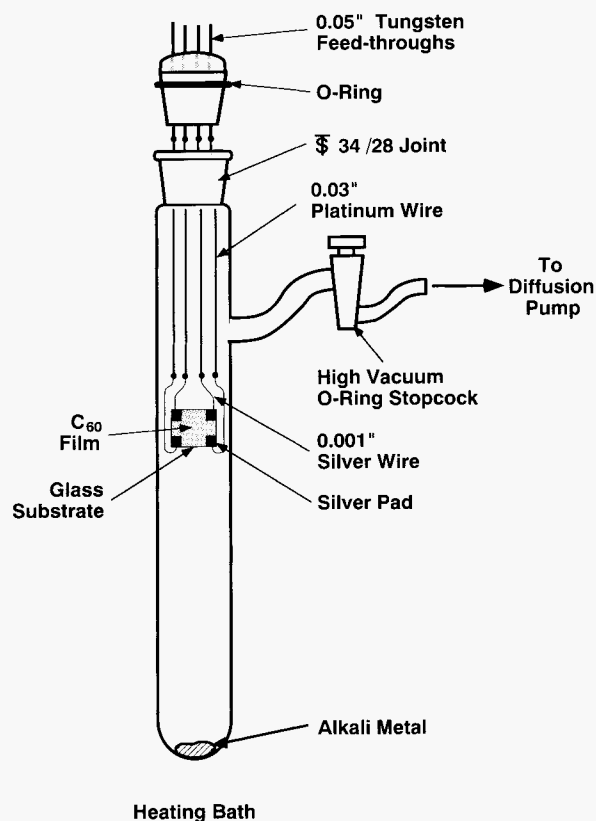


FIG. 1 Apparatus for vapour-phase doping of C_{60} and C_{70} films with alkali metals.

During the doping process, this film maintained excellent surface quality. We measured the Raman spectra of this film *in situ* (Fig. 2). The pristine film (Fig. 2a), shows the expected feature¹⁴ at $1,467\text{ cm}^{-1}$, which has been assigned to an A_g mode of C_{60} . In the highly conducting state (Fig. 2b), this line shifts to $1,445\text{ cm}^{-1}$. In the highly doped, insulating state the spectral feature moves to $1,430\text{ cm}^{-1}$ (Fig. 2c). We assign these two latter vibrations to the corresponding A_g mode in reduced forms of C_{60} . Addition of electrons to the C_{60} framework is expected to soften the bond stretching modes as the added electrons enter antibonding molecular orbitals⁷. To provide a calibration of the frequency shifts, we measured the Raman spectrum of $(\text{Ph}_4\text{As}^+\text{C}_{60}^-) \cdot 2(\text{Ph}_4\text{As}^+\text{Cl}^-)$. This salt is grown by solution electrochemistry and is known to contain the C_{60} mono-anion¹³. The Raman peak occurred at $1,461\text{ cm}^{-1}$ in this salt—a shift of 6 cm^{-1} relative to the neutral film. The lowest unoccupied molecular orbital of C_{60} is the triply degenerate t_{1u} level⁷, and the shift of 37 cm^{-1} in the Raman line of the insulating C_{60} film (Fig. 2c) therefore suggests that this state might correspond to C_{60}^{6-} , in which the t_{1u} level is completely filled. The conducting state may then be associated with energy bands derived from the partially filled t_{1u} level. A comparison may be made with graphite, in which the intralayer Raman mode at $1,582\text{ cm}^{-1}$ is shifted to $1,547\text{ cm}^{-1}$ in the intercalation compound KC_8 (ref. 15). Shifts in the Raman bands of C_{60} adsorbed on gold have also been reported¹⁶. The doped C_{60} film rapidly returned to its pale yellow colour on exposure to the atmosphere, and a Raman spectrum of this film showed that the $1,467\text{-cm}^{-1}$ line from neutral C_{60} had been restored. These observations suggest that the molecular integrity of C_{60} is maintained on doping and that the process is chemically reversible.

To ensure that the enhanced conductivity was not due to the deposition of an alkali-metal film on the surface of the C_{60} and C_{70} films, we ran a blank using two substrates each prepared with two silver contact stripes. One substrate was used as prepared, whereas the other had a C_{60} film deposited onto it as before. The two slides were placed side by side in the same apparatus with potassium as dopant. On heating, the C_{60} film first became conducting and then insulating as before, whereas the blank remained insulating throughout.

It should be noted that the materials are synthesized under non-equilibrium conditions as the experiments are performed

in a dynamic vacuum, and the local vapour pressure of the alkali metal is unknown. The rate and extent of reaction will depend on the nature of the alkali metal, the temperature of the film and the presence of residual ambient gas impurities, which are not controlled in these preliminary experiments. At present, the effect of these variables on the conductivities cannot be assessed. Synthesis in a closed system will be required to determine the relevant thermodynamic parameters.

We attribute the conductivity of the doped films to population of energy bands formed from the C_{60} π -orbitals¹⁷. As these bands become filled at high doping levels the material becomes insulating. Based on the energy-level structure of C_{60} , it is likely that the conductor–insulator transition occurs at the point where each molecule has accepted about six electrons, thereby filling the t_{1u} level⁷. We intend to carry out *in situ* X-ray, Hall effect, reflectivity and low-temperature studies on the intercalated films, as well as studying ways in which to measure and optimize the doping level and to improve the film quality. Encapsulation of an appropriate dopant within the fullerene cage could lead to increased chemical stability at the optimal doping level. □

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Faceted crystal growth in two dimensions

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CRYSTAL growth has attracted interest for centuries¹. Three-dimensional crystals are usually faceted, but equilibrium thermodynamics prohibits faceting in two dimensions²: the one-dimensional perimeter of a two-dimensional crystal cannot exhibit long-range order at any non-zero temperature³. This need not, however, prevent facets from being stable dynamically during the growth process. Computer simulations have indeed produced nearly faceted two-dimensional crystals^{4,5}. Here we describe the results of experiments on monolayers of a surfactant, sodium dodecyl sulphate (SDS), at the surface of an aqueous solution. Surface-tension measurements and fluorescence microscopy^{6–8} reveal a solid–liquid transition in the surface monolayer at fixed SDS bulk concentration, as the temperature is decreased. At low SDS concentration, faceted monolayer crystals appear, although increasing the concentration induces a change to smoother growth morphologies. The faceted crystals become unstable as growth proceeds, the corners emitting filaments of various shapes. Some of these growth processes seem not to have three-dimensional analogues.

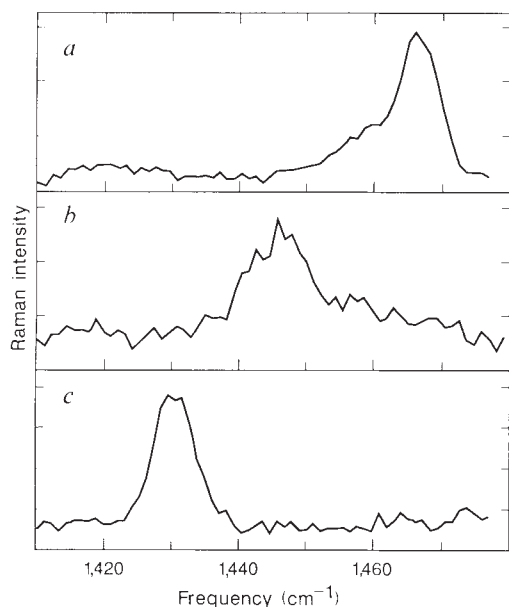


FIG. 2 *In situ* Raman spectra of a C_{60} film taken during potassium doping. a, Initial (pristine) film; b, Conducting film; c, Insulating film (see text).

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Solutions of SDS in water have been studied for a long time^{9,10} but little is known about the monolayer of SDS that is formed at the surface. Unlike the case for insoluble surfactants, the number of surface molecules in this phase is not constant: its chemical potential is determined by the bulk dissolved phase. The SDS (better than 99% pure, Fluka Chemie AG, CH-9470 Buchs, Switzerland) was first recrystallized overnight from an anhydrous methanol solution¹¹. It was then dissolved in a 10^{-3} M solution of NaCl in ultrapure water, in concentrations c ranging from 0.035% to 0.20% by weight. The critical micelle concentration is 0.25% by weight and the Kraft point (below which three-dimensional crystals may form^{12,13}) is 9 °C, both being far from our experimental conditions.

Surface tensions were measured using the Wilhelmy plate method¹⁴. The sensor was a hydrophilic membrane (Millipore Corporation, Bedford, Massachusetts, USA) attached to a stable force transducer (model 403A, Cambridge Technology Inc., Watertown, Massachusetts, USA). The two-dimensional liquid-solid phase transition appears as a change in slope of a plot of lateral pressure against temperature (Fig. 1). The location of the transition is generally in good agreement with direct visual observation of the melting point. Figure 2 shows the liquid-solid transition in the c - T plane as observed both visually (circles) and from surface-tension measurements (crosses).

The fluorescence apparatus has been described elsewhere¹⁵. Samples (2 ml) of the SDS solution were pipetted into a square Teflon trough (3.5×3.5 cm), and a small quantity of dye (an insoluble fluorescent surfactant: 12-NBD-stearic acid, 98% pure, Molecular Probes Inc., Eugene, Oregon, USA) was spread onto the surface. The surface density of dye was 10^{-2} molecule per nm^2 , compared with the 5 molecules per nm^2 needed for a full monolayer of single-chain molecules. Because the SDS concentration was lower than the critical micelle concentration, the dye did not dissolve into the bulk phase. The solution was thermally regulated with a Peltier element. In a typical run, temperature was decreased at a constant rate, with the film initially liquid-like. Crystals appeared as dark regions on a brighter background, as the dye was expelled during the growth process. As we were working far from the stability region for three-dimensional crystals (Fig. 2), the surface film was two-dimensional.

Figure 3 shows the different morphologies obtained for slow changes in temperature at three different SDS concentrations. For $c = 0.055\%$ (Fig. 3a), faceted hexagonal crystals, usually with defects, were obtained, with facets as large as $50 \mu\text{m}$. At a higher concentration, $c = 0.12\%$, smoother but still anisotropic morphologies were found (Fig. 3b). For $c = 0.17\%$, growth was isotropic. When dodecanol impurities were present in the system, the interface of these circular crystals underwent a Mullins-

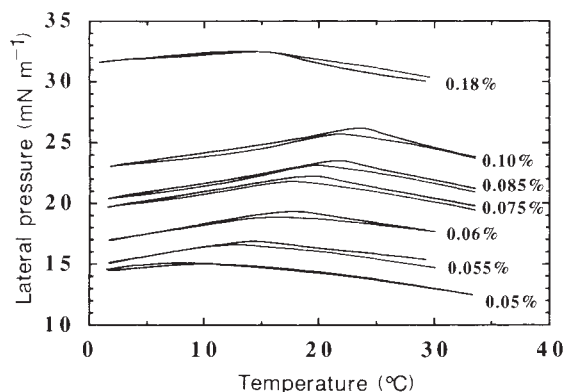


FIG. 1 Lateral pressure as a function of temperature for different SDS bulk concentrations. Each curve represents a temperature cycle. Lateral pressure is defined as $\sigma_w - \sigma$, the difference between σ_w (surface tension of pure water) and σ (surface tension of the SDS solution).

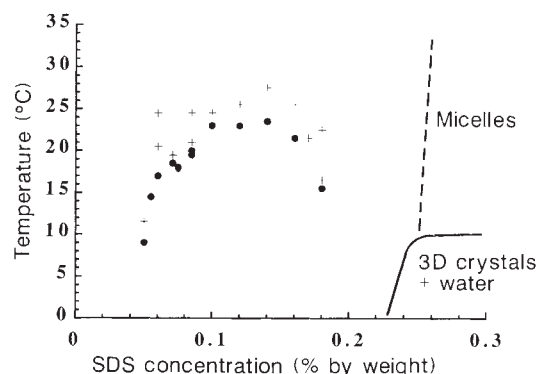


FIG. 2 Phase diagram in the concentration-temperature plane, showing the experimental liquid-solid phase boundary. Crosses are from thermodynamic measurements of surface tension, circles represent observations from fluorescence microscopy. The monolayer is solid below the transition line and liquid above. The stability regions are also shown for micelles and three-dimensional crystals in water.

Sekerka branching instability¹⁶. The transition between sharp and rounded corners occurs close to $c = 0.10\%$, the resolution being too low for more precise characterization. Figure 1 shows that facets grow at low two-dimensional pressures (below $\sim 25 \text{ mN m}^{-1}$), possibly below the triple point for the SDS monolayer. The morphology change between faceted and smooth shapes may be related to the nature of the fluid (gas or liquid) in which the crystal grows. This would imply a change in slope of the solid-liquid transition line in Fig. 2 at around $c = 0.10\%$ (at which the morphology changes), where an (invisible) liquid-gas transition line would start. More experiments are needed to elucidate this point.

At some point during the growth process, facets became unstable, the corners developing instabilities of various shapes. Figure 4a shows one such instability, in which zigzag branches

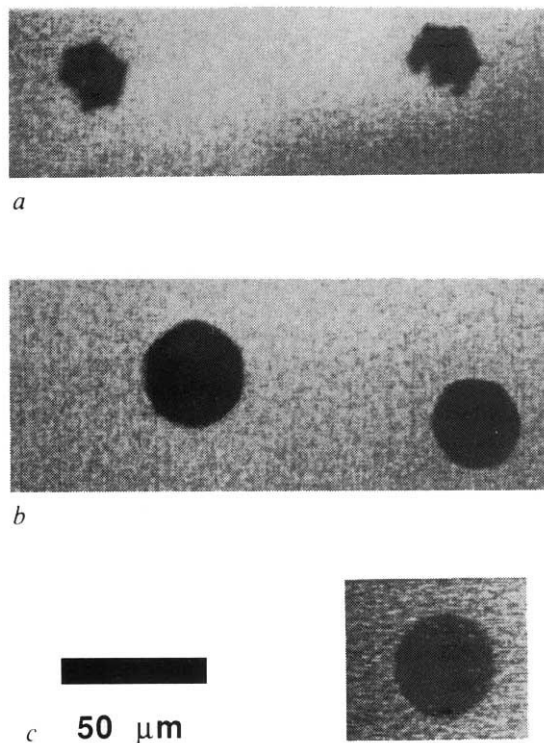


FIG. 3 Morphologies of crystals obtained on decreasing the temperature slowly. A, $c = 0.055\%$. B, $c = 0.12\%$. C, $c = 0.17\%$.

emanated from each corner. The direction of propagation and the periodicity of the zigzag modulation are well defined, suggesting that diffusion of rejected impurities plays an important role^{17,18}. To our knowledge, no analogous phenomenon exists in three dimensions. Figure 4b shows another situation, in which corners emit linear filaments which eventually split in two at an angle of 120°. The filaments connect with those on neighbouring crystals while continuing to lengthen, causing them to curl into the pattern shown in (Fig. 4c). The usual explanation for the growth of thin whiskers in three dimensions relies on the existence of screw dislocations, along which growth is very fast¹⁹. Clearly this explanation cannot be transposed to two dimensions; another interpretation is required. Other patterns could be obtained by varying parameters such as the bulk SDS concentration, dye concentration, cooling rate, pH, conductivity and so on. Further experiments are needed to investigate these dependences systematically.

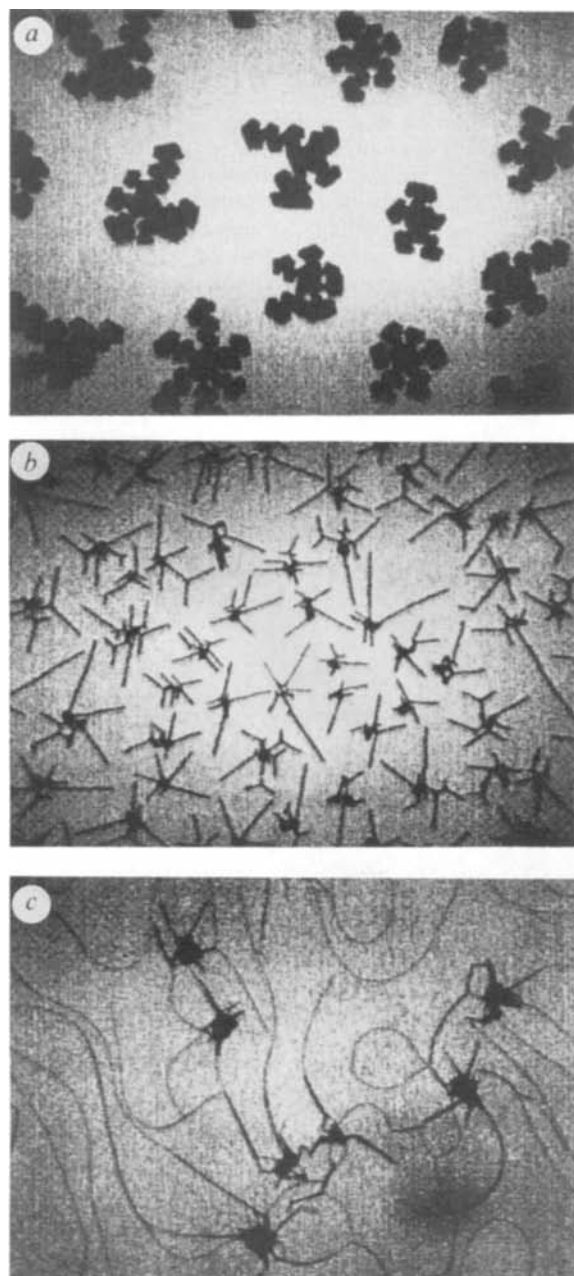


FIG. 4 Three types of instability observed during growth of faceted crystals. Each frame is 650 μm $\times$ 450 μm .

These observations raise a number of questions. If they cannot be explained by equilibrium thermodynamics, a mechanism is required for dynamically stable faceted growth which allows a continuous transition from angular to smooth shapes. It is not clear whether or not the numerical simulations^{4,5} that show this kind of behaviour provide a realistic model of this system. Others experiments^{6-8,20-22} also show an unexpected variety of shapes in two-dimensional growth processes, but the underlying physics of these processes remains elusive. $\square$

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Interdecadal oscillations and the warming trend in global temperature time series

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THE ability to distinguish a warming trend from natural variability is critical for an understanding of the climatic response to increasing greenhouse-gas concentrations. Here we use singular spectrum analysis¹ to analyse the time series of global surface air temperatures for the past 135 years², allowing a secular warming trend and a small number of oscillatory modes to be separated from the noise. The trend is flat until 1910, with an increase of 0.4 °C since then. The oscillations exhibit interdecadal periods of 21 and 16 years, and interannual periods of 6 and 5 years. The interannual oscillations are probably related to global aspects of the El Niño–Southern Oscillation (ENSO) phenomenon³. The interdecadal oscillations could be associated with changes in the extratropical ocean circulation⁴. The oscillatory components have combined (peak-to-peak) amplitudes of >0.2 °C, and therefore limit our ability to predict whether the inferred secular warming trend of 0.005 °C yr⁻¹ will continue. This could postpone incontrovertible detection of the greenhouse warming signal for one or two decades.

Over the past decade, substantial efforts have gone into establishing reliable and accurate records of surface air temperatures for periods of a century or more^{2,5-10}. The Intergovernmental Panel on Climate Change (IPCC) Report¹⁰ provides an excellent review of remaining problems. We have chosen for the present analysis the time series of annually-averaged temperatures from 1854 to 1988 produced by the Climate Research Unit (CRU) of

the University of East Anglia^{2,8}, and verified the results against the IPCC consensus time series¹⁰ (1856–1989).

Only the annual means for the Northern Hemisphere (NH), Southern Hemisphere (SH) and the entire globe were used here. Various corrections cited in chapter 7 of ref. 10 are $\sim 0.1^\circ\text{C}$ for global temperatures, and refer to changes occurring in the data base over many decades.

Kuo *et al.*¹¹ have reviewed the pitfalls involved in using parametric statistical methods for studying trends in short time series. They proposed a semi-parametric method, in which the trend itself is fitted by a straight line, depending on two parameters (the average and the slope), and the residue of this fit is analysed by the non-parametric multi-taper method^{12–15}. The rise in global temperature since the mid-nineteenth century is far from uniform, however, as inspection of temperature records^{2,5–10} immediately reveals. We have therefore applied a method which is fully non-parametric with respect to both the trend and residue. The method, singular spectrum analysis (SSA), is fully data-adaptive, and has previously been introduced in ref. 16 and ref. 17.

SSA considers M lagged copies of a centred time series $X(t)$ sampled at equal intervals τ_s , $X_i = X(t_0 + i\tau_s)$, $1 \leq i \leq N$, and calculates the eigenvalues λ_k and eigenvectors $\mathbf{p}_k$ of their covariance matrix $\mathbf{C}$, $1 \leq k \leq M$. By analogy with nomenclature from the meteorological analysis of random fields¹⁸, Vautard and Ghil¹ (VG hereafter) called the eigenvectors empirical orthogonal functions (EOFs), and the coefficients $\mathbf{a}_k$ involved

in the expansion of each lagged copy of X , principal components (PCs). The expansion is as follows:

$$X_{i+j} = \sum_{k=1}^M a_{ki} p_{kj} \quad (1a)$$

$$a_{ki} = \sum_{j=1}^M X_{i+j} p_{kj} \quad (1b)$$

In our application $N = 135$ and $M = 40$.

VG observed that pairs of high-variance eigenvalues $\lambda_k \approx \lambda_{k+1}$ are associated with oscillatory phenomena: both the corresponding EOFs and PCs are in quadrature with each other. The corresponding EOFs can be thought of as a data-adaptive, anharmonic sine-and-cosine pair. This observation is rigorously true only for a pure oscillation¹, but holds in practice for the palaeoclimatic marine records studied by VG, the Vostok isotopic temperature record¹⁵ a 38-year record of mean-monthly values of zonal wind at the Equator³ and the leading PCs of a spatial EOF analysis of geopotential heights¹⁹ taken daily at 700 mb (NH) and 500 mb (SH).

These pairs are interesting only when they stand out above the nearly-constant noise level^{16–19}. A stochastic model (R.V. and M.G., manuscript in preparation) was used to find the smallest number S of deterministic modes consistent with the CRU data².

Figure 1a shows the eigenvalues λ_k , in decreasing order of variance. The first 12 eigenvalues account for 83% of the total variance; the stochastic model shows that the time series has $\sim S = 12$ significant components—this is the smallest S for which no eigenvalues fall outside the 95% confidence interval.

Figure 1b shows the first four EOFs of the time series. The first is a data-adaptive set of weights for a running mean (ref. 1 and equation 1b). As $\mathbf{C}$ is a Toeplitz matrix, all EOFs are either symmetric or antisymmetric¹. The first EOF is symmetric, the second one antisymmetric; the first two PCs represent the trend in the data (Figs 3 and 4), and together account for 62% of the variance. EOFs 3 and 4 are an oscillatory pair ($\lambda_3 \approx \lambda_4$): they are in quadrature and have a period of ~ 20 years.

The window width $\tau_w = M\tau_s$ represents a compromise between significant information and statistical confidence. Given the relatively long periods apparent from the instrumental temperature records^{2,5–10} and illustrated here by EOFs 3 and 4, M values much lower than 30 would miss important information; values much larger than 50 would carry too little significance (fewer than three repetitions of the given phenomenon in the data). Values of $30 \leq M \leq 50$ do not substantially alter the results of Fig. 1, or the subsequent results in Figs 2–4.

Additional oscillatory pairs (not shown) are given by EOFs 6–7 and 11–12. The triplet 8–10 also carries an oscillation with a period of ~ 9 years. To ascertain further the period of the EOFs, the power spectrum of each PC was calculated by the non-parametric multi-taper method^{11–15} (not shown). The results are summarized in Fig. 2. The filtered and raw spectra agree fairly well down to periods of ~ 5 years, showing that SSA performs as a non-parametric, data-adaptive low-pass filter.

Fundamental oscillations are associated with the peaks near 21 years (pair 3–4), 6 years (pair 11–12) and 5 years (pair 6–7). Peaks near 21 and 16 years also appear in a maximum-entropy analysis of global marine air temperatures and sea surface temperatures⁵. A peak at 23 years dominates the maximum entropy spectrum of a 300-year Central England Temperature record²⁰, whereas the Fourier transform²¹ of global marine air temperature data⁹ contains a 21.8-year peak, the sixth harmonic of the length of record used there (135 years). A period of 23 years was found in the seasonal distribution of rainfall at Adelaide²², as well as at Cape Town and Santiago (R. Hide, personal communication based on joint work with R. A. Fisher in the 1960s).

The 5- and 6-year peaks (pairs 6–7 and 11–12) are likely to be associated with the low-frequency component of ENSO^{3,30}.

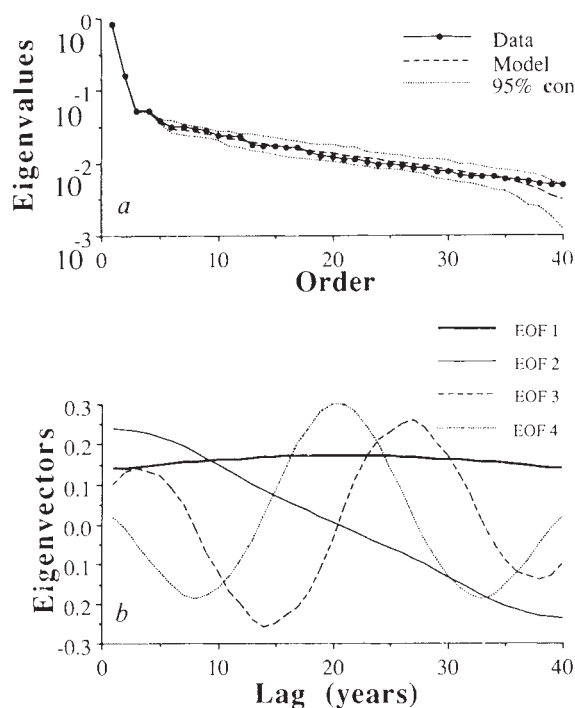
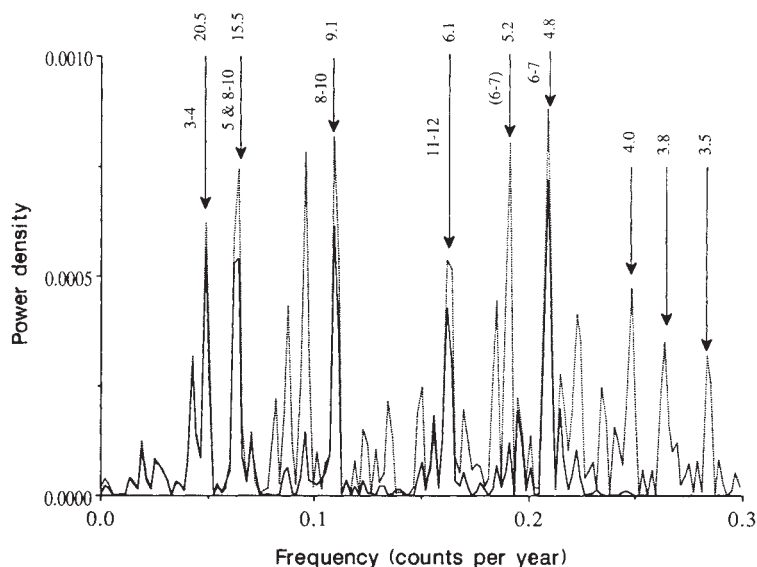


FIG. 1 a, Spectrum of the lag-covariance matrix $\mathbf{C}$ of the time series of global surface temperatures². The heavy dots are the calculated eigenvalues λ_k (united by a solid line); the dashed line indicates the mean spectrum of the stochastic model using $S=12$, the dotted lines upper and lower bounds for 95 out of 100 Monte Carlo realizations (the eigenvalues of only two realizations fall below and two above the corresponding dotted lines). The model consists of adding $M-S$ random modes of equal variance to the S leading modes whose PCs agree with those computed by equation 1b to within the (constant) white-noise level. This model is consistent with the results of a non-parametric Kolmogorov–Smirnov test, which shows that the time series is stationary with a confidence of 95%—the distributions for two halves of the series are indistinguishable no matter where the halving point is taken (except near either end). b, The four leading eigenvectors, $\mathbf{p}_1$ – $\mathbf{p}_4$; the first two are associated with 62% of the variance, the next two with 7%.

FIG. 2 Multi-taper method power spectra of the temperature time series²: the trend has been removed non-parametrically by summing only over the first two PCs ($k=1, 2$) in equation 1a (see equation 2 in the text). The dotted line is the detrended raw data, the solid line data from which both trend and noise have been removed. All peaks labelled with the corresponding period (in years) are significant in the raw (detrended) data at the 97% level; numbers along the arrows indicate which significant PCs contribute to the peaks. The multi-taper method provides an F -test for the significance of a spectral peak with results which, unlike those for the Blackman-Tukey method, are not directly proportional to the amplitude of the peak^{13,15}. All the peaks marked by arrows have identical frequencies in both hemispheres, and pass the F -test at the 97% level in each hemisphere separately; the only exception is the pair 5.2 years (NH) and 4.8 years (SH). The window width used for all four time series (global raw and filtered, NH and SH filtered; all detrended) is $2BN=6.0$, with eight leakage-resistant tapers, where $N=135$ and B is a roll-off parameter of the method. Differences when using a window of 1.5 and four tapers or a window of 3.0 and six tapers are small.



The separation between a high-frequency, quasi-biennial component of ENSO and a low-frequency, 5–6 year component obtained in tropical wind data³ has been confirmed by an analysis of 108 years of monthly values of the Southern Oscillation Index²³.

The most interesting aspect of SSA is the detailed reconstruction of any subset $\mathcal{H}$ of significant components of the time series. The optimal linear combination of the corresponding PCs giving least-square deviation from the original data is, for this subset (R.V. and M.G., manuscript in preparation),

$$X_i^{\mathcal{H}} = \frac{1}{M} \sum_{j=1}^M \sum_{k \in \mathcal{H}} a_{k,i-j} \rho_{kj} \quad (2)$$

For instance, if $\mathcal{H} = \{1, 2, \dots, S\}$, the reconstruction involves, in the precise sense defined above, the statistically-significant

part of the time series. Using only the two PCs and EOFs involved in a pair gives the evolution in time of the amplitude and phase for the nonlinear oscillation they represent^{1,19}, in much greater detail than complex demodulation for classical Fourier analysis. This optimal reconstruction has been used in Figs 3 and 4 below, as well as for removal of the trend in the time series analysed spectrally in Fig. 2.

In Figs 3a and b, the secular trend (based on the reconstruction from the first two PCs) is the heavy solid line. In good agreement with the evaluation of Jones *et al.*², global temperatures show no trend until 1910, then marked warming to about 1940, a flattening out to about 1975 and an upward trend since then. The bi-decadal oscillation superimposed on this trend (based on PCs 3–4) is most in evidence during the times of little systematic rise in temperatures (1870–1910 and 1940 onwards).

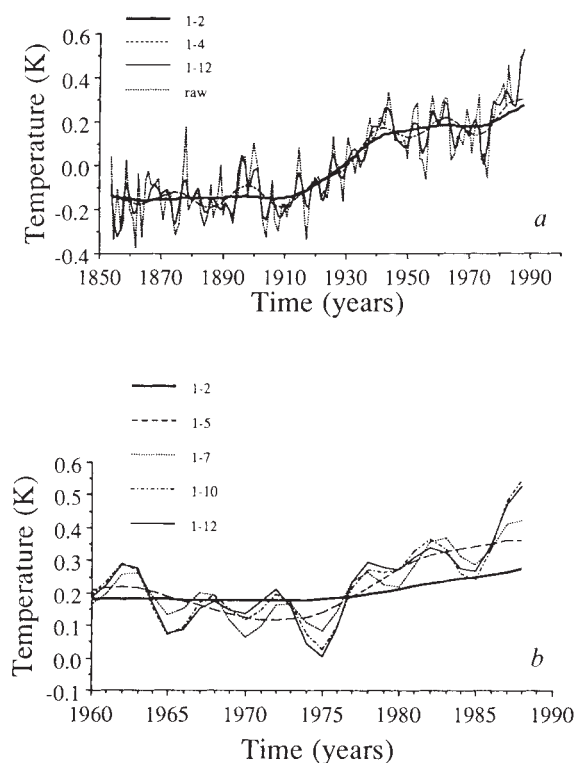
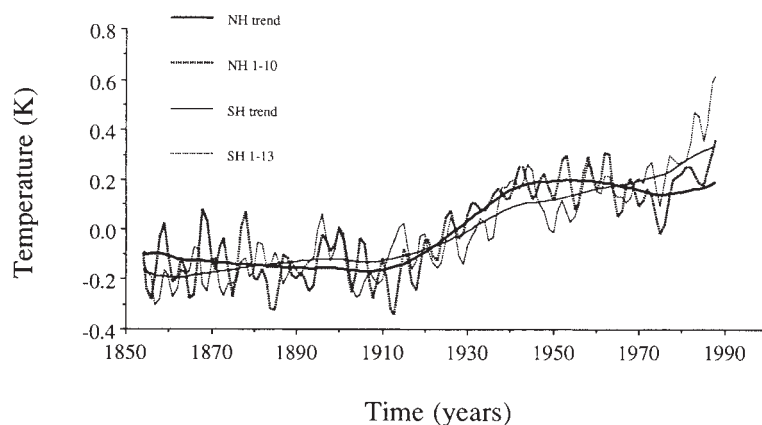


FIG. 3 Reconstruction of the secular trend (PCs 1–2), and of the interdecadal (PCs 3–4) and interannual (PCs 6–7, 11–12) oscillations in global temperature². The different reconstructions are labelled on each panel. *a*, Entire time series; *b*, past 30 years. The trend differs from a running mean, as given by PC1 alone; the additional use of PC2 is more accurate for low-frequency filtering, especially for frequencies near $f_w = \tau_w^{-1}$. At the beginning and end of the time series, $1 \leq i \leq 0.5M$ and $N - 0.5M \leq i \leq N$, the summation in equation 2 is restricted to the available PC data, so that averaging occurs over fewer and fewer lags as the ends are approached.

FIG. 4 Reconstruction of the trend and filtered part of the time series for NH (heavy solid and heavy dots) and SH (light solid and dots) temperatures².



The global IPCC time series shows no significant differences from the CRU data² in the characteristics of either the interdecadal or the interannual oscillations. The IPCC trend is slightly smaller in amplitude than that shown here, although the rapid rise occurs at the same time.

The part of the time series reproducible in both data sets^{2,10} (PCs 1–12) shows large excursions about the trend. It is striking that it has been above the trend since 1976, and as high above it in 1988 as at any time in the past (even higher than at the turn of the century). The raw data² differ from the filtered data by high-frequency fluctuations (compare with Fig. 2) of 1–2 years duration; the amplitude of the difference is especially high in the earlier half of the time series (for example 1878), probably because of the poorer quality of the data then. But large 'random' fluctuations still occur later than 1950; some of these might be due to the quasi-biennial component of ENSO^{3,23}, which almost coincides with the Nyquist frequency of our data.

After removal of the trend (PCs 1 and 2), 55% of the variance is quasi-periodic and therefore predictable. In Fig. 3b, we can inspect in detail the contribution of the oscillatory components during the last 30 years. The bi-decadal oscillation was near its maximum in 1988. The larger of the two low-frequency ENSO pairs (6–7) was also at its maximum, while the smaller (11–12) was at zero on its way down. The component of the signal corresponding to the peak around 9 years in Fig. 2, represented by the triplet of EOFs 8–10, is also near its maximum. Extrapolation of this oscillatory behaviour indicates that global temperatures are likely to decrease over the next decade.

The phase of the reconstructed time series is estimated less well near the ends of the time series. Nevertheless, detailed prediction for about one-quarter to one-third of the window width τ_w can be based on a combination of SSA and maximum entropy methods^{23,24}, and has been applied successfully to the Southern Oscillation Index²³. Its application to the temperature data here is left for a separate publication.

Finally, we consider the differences in averaged surface air temperatures between the NH and the SH (Fig. 4). Both time series were subjected separately to SSA, yielding in both cases PCs 1 and 2 as carrying the trend, with $S \approx 10$ for the NH and $S \approx 13$ for the SH data. The trend is smoother for the SH, with a near-uniform increase since 1910, whereas the NH has experienced a temperature decrease between 1950 and the mid-1970s (compare also refs 2, 10 and 25).

The low-frequency oscillations, both bi-decadal and ENSO (separate reconstructions not shown), are fairly coherent: only some of the shortest excursions of the two dotted curves disagree. ENSO-related oscillations are coherent between the two hemispheres, as they arise in the tropical Pacific and involve exchanges of water masses with the extratropics²⁶ as well as global atmospheric effects²⁷. The coherence of the interdecadal oscillations argues for their equally global character.

A solar origin for the bi-decadal oscillation²¹ is unlikely for

three reasons. First, any insolation effects of the Sun's 22-year activity cycle should be dominated by the rectified 11-year period, which is absent from our analysis. Second, the multi-channel SSA analysis of North-American temperature data⁷ reveals no coherence with the 11-year insolation signal (M. Dettinger, personal communication). Third, although the 0.1% amplitude of the solar irradiation cycle would be sufficient to cause variations of 0.1 °C in atmospheric temperatures, it is definitely not sufficient to heat the upper ocean down to the penetration depth of a decadal signal²⁸.

The interdecadal oscillations with a period of about 21–23 years could involve water masses below the seasonal thermocline and outside the tropics. Bjerknes's⁴ pioneering work on interdecadal oscillations in the North Atlantic climate has found recent support in the work of Levitus²⁹, which shows temperature and salinity changes down to the permanent thermocline on this time scale. Modelling of coupled ocean-atmosphere oscillations on the interdecadal time scale is in progress. □

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Rapid disintegration of the Wordie Ice Shelf in response to atmospheric warming

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THE breaking up of ice shelves around the Antarctic Peninsula has been cited¹ as a "sign that a dangerous warming is beginning in Antarctica". Here we present satellite images showing the disintegration of the Wordie Ice Shelf, which lies off the west coast of the Antarctic Peninsula (Fig. 1). Fracture, either in the form of surface crevasses or rifts extending to the bottom of the ice shelf, has been responsible for iceberg calving and weakening the central region of the ice shelf. These fracture processes, which led to retreat of the ice front, were apparently enhanced by the presence of increased amounts of melt water, resulting from a warming trend recorded in mean annual air temperatures in Marguerite Bay. If this warming trend continues, other nearby ice shelves on the Antarctic Peninsula may be at risk. But substantial additional warming would be required before similar processes could initiate breakup of the Ross and Filchner-Ronne ice shelves, which help stabilize the West Antarctic ice sheet.

We have used Landsat images of the whole ice shelf taken in 1974, 1979 and 1989 (Fig. 2) and images covering only the ice front portion taken in 1986 (Landsat) and 1988 (SPOT). The 1989 image was registered to surveyed ground control points and used as a base to which the other images were registered and resampled. Features in separate images could be identified and matched as far as pixel size (25 m) allowed, and their positions compared with an accuracy of ~50 m. Using the position of the ice front in 1966 mapped from aerial photography, we estimate that the area of the ice shelf has decreased from ~2,000 km² in 1966 to ~700 km² in 1989 (Fig. 3). But defining the position of the ice front can be very uncertain; large blocks that calve off to form icebergs are difficult to classify as being either attached to, or separated from, the ice shelf.

Before about 1979, the retreat of the ice front occurred mainly by transverse rifting, creating icebergs up to 10 km by 1 km. The western area was the first to be lost, between 1966 and 1974. Results from airborne radio-echo sounding between 1966 and 1970 indicated that the ice shelf could be divided by the 67°50' W meridian into a crevassed eastern part and a rifted western part where brine welling up could infiltrate the ice at sea level². The 1974 image shows many transverse rifts south of Napier Ice Rise, whereas by 1979 longitudinal rifting was predominant. Many longitudinal rifts had formed upstream of Buffer, Linchpin and Napier Ice Rises by 1979, some following pre-existing flow lines. A critical factor in the breakup was the decoupling of Buffer Ice Rise; by 1986 ice was streaming past it apparently unhindered, in contrast to the compressive upstream folding seen in earlier images. Between 1988 and 1989 the central part of the ice shelf, consisting of broken ice in the lee of Mount Balfour, was lost, exposing the coastline and effectively dividing the ice shelf in two. By 1989, longitudinal rifting had penetrated to the grounding line (where ice flowing off the land begins to float in the sea) north of Mount Balfour. Further north, a growing shear zone marked the boundary between fast-flowing ice from the north Forster Ice Piedmont and the slower flow of ice from Hariat Glacier.

We have derived velocities at more than 70 sites near the ice front by comparing the positions of matched features on the 1986, 1988 and 1989 images. There are three distinct regions, corresponding to the main glacier inputs³: ice from Hariat Glacier has velocities of ~200 m per yr; the tongue from the northern part of Forster Ice Piedmont increased in velocity from ~1,800 m per yr between 1986 and 1988 to reach 2,000 m per

yr or more in 1988/89; and the tongue from Prospect Glacier and the southern part of Forster Ice Piedmont had velocities of ~1,000 m per yr in 1988/89. In the northern part of the ice shelf, no features can be matched unambiguously between images taken before 1986; however, in the southern part, a comparison between the 1974 and 1979 images reveals features moving at ~600 m per yr, significantly less than the 1988/89 velocity. Increases in velocity are expected as the ice shelf retreats because restraint from ice rises and ice rumple is reduced. It has not been possible to derive velocities near the grounding line, but we have detected no changes in flowline position on the input glaciers. This suggests that changes in input have not been responsible for breakup, nor have the effects of breakup propagated far inland. The velocity of Fleming Glacier has been surveyed at a site about 50 km upstream from the grounding line^{4,5}; in 1975 it was ~200 m per yr and remeasurement would show if there had been any significant change.

The major ice rises have had several roles in controlling the behaviour of the ice shelf. Whilst embedded in the ice shelf, they created broken wakes downstream and zones of compression upstream which helped to stabilize the ice shelf. During the retreat of the ice front, they temporarily pinned the local ice front position and also acted as nucleating points for rifting which quickly stretched upstream, indicating that a criterion for fracture had been exceeded. At this stage, an ice rise, instead of protecting the ice shelf against decay, aided its destruction by acting as an indenting wedge. Fracture of ice is a critical process in ice-shelf dynamics⁶. Before we can understand ice-shelf retreat, either of the Wordie in the past or of other ice shelves in future, mathematical models based on continuum mechanics^{7,8} will have to incorporate fracture mechanics to describe iceberg calving and how ice rises can initiate fracture both upstream and downstream.

Ice shelves along the west coast of the Antarctic Peninsula exist up to a climatic limit¹, taken to be a 0 °C summer isotherm. Wordie Ice Shelf is the northernmost ice shelf of any note in this region⁹ and might therefore be expected to be sensitive to changes in its environment, such as atmospheric or oceanic warming. In Marguerite Bay, there are too few sea temperature measurements to show whether warming has increased basal melting¹⁰, but mean annual air temperatures since 1962 have fluctuated between -8.6 °C and -1.8 °C, the warmest being recorded¹¹ in 1989. The temperature record shows warm spells in the early 1970s and mid-1980s, with increasing temperatures from 1987 to the present.

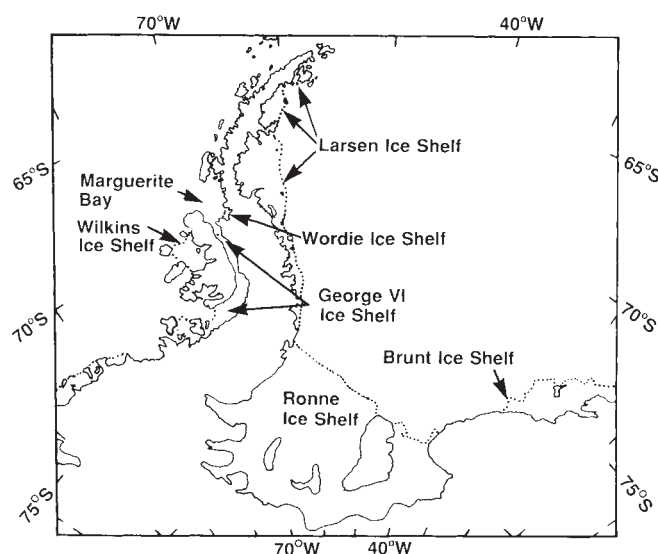


FIG. 1 Map of Antarctic Peninsula showing location of Wordie Ice Shelf.

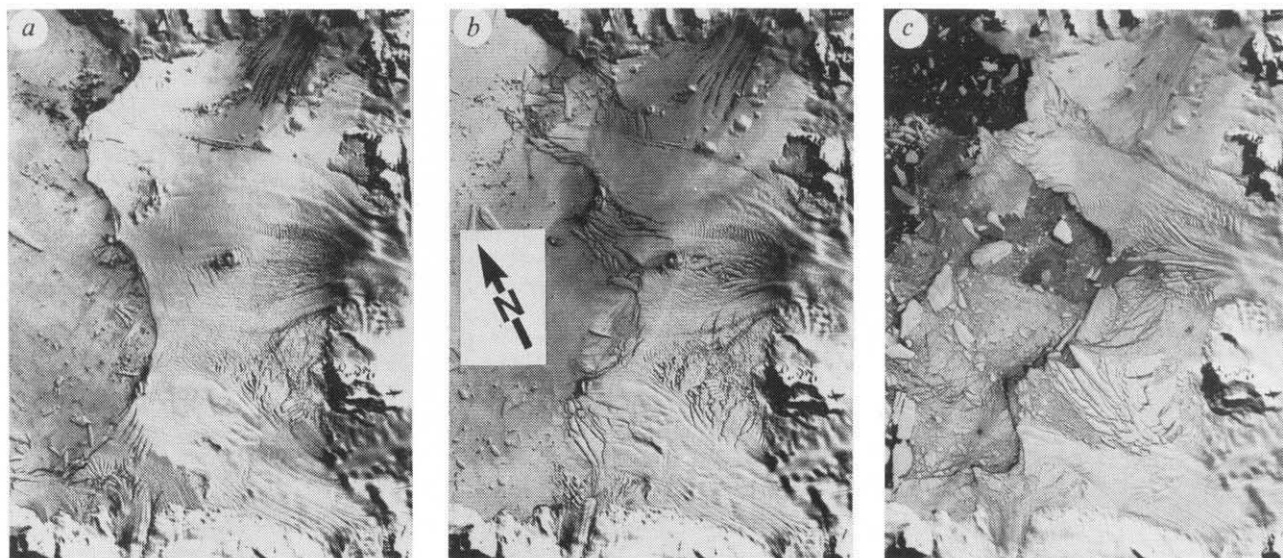


FIG. 2 Landsat images of Wordie Ice Shelf taken on *a*, 6 January 1974, *b*, 3 February 1979 and *c*, 20 February 1989 (published by courtesy of EOSAT).

The images cover an area of $\sim 45 \text{ km} \times \sim 65 \text{ km}$, which is shown by the bold outline on Fig. 3. There is a north-pointing arrow on *b*.

Several algorithms relating mass balance parameters (precipitation and ablation) to mean annual air temperature have been used for climate modelling^{12,13}. For temperatures higher than -12°C the net annual accumulation, N , can be expressed as a function of the precipitation, P , the mean annual temperature T and a parameter, m , related to the ablation rate by

$$N = P - m(12 + T)^n$$

where $n = 1$ for a linear model¹² or $n = 2$ for a quadratic model¹³, and P has been assumed for simplicity to be independent of T over the relatively small temperature range of interest here. We used these mass balance models with the mean annual temperature record for Marguerite Bay to construct net-accumulation curves by summing over the period of the record (1962–1989). Lack of data means that reliable values for the model coefficients P and m cannot be derived, so they were chosen to make the net-accumulation curves show a near-zero response during the 1960s. Results were very similar for both the linear and nonlinear models, and suggested that there have been several periods of substantial melt during the warm periods, with net ablation of several metres since 1970. The models show that the ice shelf lies in a sensitive region where a small change in temperature could cause a large change in the net balance. The critical temperature, below which the net accumulation is positive (mass gain) and above which it is negative (mass loss) is about -5°C .

We suggest that breakup was triggered by a climatic warming that increased ablation and the amount of melt water. Laboratory experiments show that the fracture toughness of ice is reduced at higher temperatures and possibly by the presence of water^{14,15}. Instead of refreezing in the upper layers of firn, free water could percolate down into crevasses and, by increasing the pressure at the bottom, allow them to grow into rifts¹⁶ or possibly to join up with basal crevasses¹⁷. Processes such as these would increase the production rate of blocks above that required for a 'steady state' ice-front position. The blocks will drift away as icebergs if conditions, such as bay geometry and lack of sea ice, are favourable. Thus, ice-front retreat would be a sensitive function of mean annual air temperature. Some ice shelves, like Brunt, are formed from blocks that break off at the coastline and, unable to float away, are 'glued' together by sea-ice and snow-fall¹⁸. These heterogeneous ice shelves contrast with those such

as Ronne, where glaciers or ice streams flow unbroken across the grounding line to form a more homogeneous type of ice shelf. Before 1989, Wordie Ice Shelf consisted of a mixture of both types, the main tongues being derived from glacier inputs, whereas the central portion was formed from blocks breaking off at the grounding line and at the sides of the main tongues. The western rifted area that broke away between 1966 and 1974 was described by Swithinbank, who flew over it in early 1967, as "snowed-under icebergs" (ref. 19), and it was the heterogeneous central part which broke back to the coastline around Mount Balfour in 1988/89. Increased ablation would not only enhance rifting along lines of weakness but would also loosen the 'glue' that held the blocks together.

Ice shelves lying close to regions of net ablation (where the mean annual temperatures are higher than about -5°C , if the mass balance models used here apply elsewhere) will be at risk

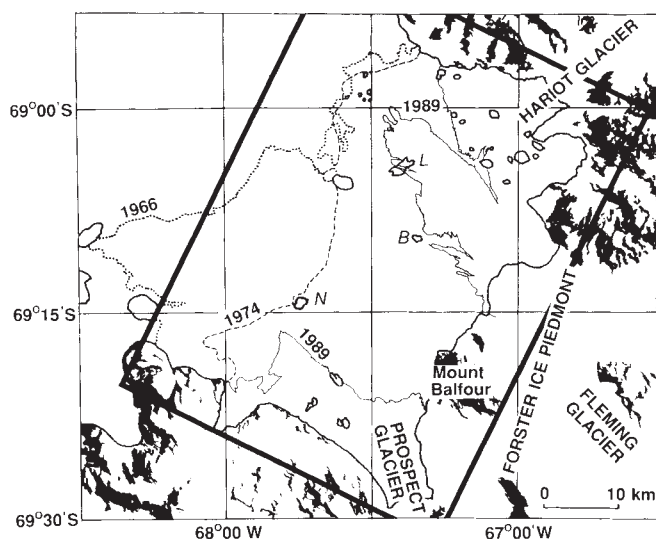


FIG. 3 Positions of ice front in 1966 (dotted line), 1974 (dashed line) and 1989 (continuous line). Lettered ice rises are: *B*, Buffer; *L*, Linchpin; *N*, Napier. The bold line depicts the area covered by the images in Fig. 2.

from the mechanism outlined above. In the Antarctic Peninsula, the northern ice fronts of George VI and Larsen ice shelves have a history of recession over the past fifty years¹⁹ and are still retreating. Another at risk is Wilkins Ice Shelf. Snow temperatures indicate that this region is at most 1–2 °C colder than the Wordie²⁰ and radio echo measurements show that large areas are infiltrated by brine. If the warming trend continues for another decade, this may be the next ice shelf to start receding. □

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Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands

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METHANE and nitrous oxide are long-lived, radiatively active trace gases that account for ~20% of the total anticipated atmospheric warming¹. The atmospheric concentrations of both gases have increased dramatically over the past few decades, and continue to increase at a rate of ~1.1 and 0.25% yr⁻¹ for CH₄ (ref. 2) and N₂O (ref. 3) respectively. Increased biospheric production is generally suggested as the reason for the increases, but decreases in global sinks may also be important. It has been suggested, for example, that nitrogen fertilization may decrease the rate at which tropical^{4,5} and temperate forest soils⁶ take up methane from the atmosphere. Furthermore, the recent extensive changes in land management and cultivation could be contributing to the observed increases in both atmospheric CH₄ and N₂O, as has been suggested for tropical soils⁷. Little information exists on CH₄ uptake in temperate grasslands (which currently occupy ~8% of the Earth's surface), its relation to N₂O production, or the effect of land management or cultivation^{8,9}. Here we report measurements of CH₄ uptake and N₂O emissions in native, nitrogen-fertilized and wheat-growing prairie soils from spring to late autumn, 1990. We found that nitrogen fertilization and cultivation can both decrease CH₄ uptake and increase N₂O production, thereby contributing to the increasing atmospheric concentrations of these gases.

We established three sets of sites in the Colorado shortgrass steppe during late winter and spring, 1990. One set was estab-

lished along a toposequence (catena) to determine whether the landscape position—sandy loam upland (midslope) versus sandy clay loam lowland (swale)—in the native prairie (catena site¹⁰) affected CH₄ uptake. Previous investigations^{10,11} had found that ammonia and N₂O fluxes differed markedly with landscape position. A second set of sites was established in unamended and annually nitrogen-fertilized native grass pastures. The third set was established in a pair of wheat fields, cropped in alternating years (wheat-fallow system), and in an adjacent native grassland for comparison. All sites were in or near the United States Department of Agriculture–Agricultural Research Service Central Plains Experimental Range located north of Nunn, Colorado (latitude 40° 48' 23" N, longitude 104° 45' 15" W). Table 1 summarizes the soil characteristics.

On the catena, the fertilized midslope site was treated with 45 g m⁻² of urea-N in July 1981, and the fertilized swale site was similarly treated in May 1982 (ref. 11). Four replicate, permanent plots for gas flux measurement were established in the fertilized and unfertilized midslope and swale sites by driving 20.3-cm diameter PVC pipes into the soil to a depth of 8 cm. Cattle have been excluded from the catena site since 1980. Weather and soil temperature data were continuously collected ~200 m from the site.

The annual fertilization experiment was ~1 km north of the catena site. We established six replicate plots in both nitrogen-fertilized and control grazed pastures, hereafter called AFS (annual fertilized) and CAFS (control annual fertilized) sites. The terrain in these two pastures is essentially flat, and the soil is similar to the catena midslope (Table 1). The AFS received 2.2 g N m⁻² of ammonium nitrate annually from 1976 to 1989.

Finally, we established six plots in each of three adjacent sites—wheat, fallow, and native grassland—6 km east of the Central Plains Experimental Range. The wheat and fallow sites had been in continuous cultivation without irrigation or application of nitrogen fertilizer, pesticides or herbicides since the native prairie was ploughed in 1981. Winter wheat was planted in September 1989, and the fallow site was kept weed-free by cultivation following harvest in July 1989. The crop was harvested in July 1990, and the fallow site was planted with wheat in September 1990. The native grassland is 200 m east of the wheat-fallow field.

We measured methane and nitrous oxide fluxes by placing a closed chamber¹² over the established plots and taking gas samples from inside the chambers with 50-ml polypropylene syringes fitted with nylon stopcocks, usually 0, 15 and 30 minutes after the chambers were installed. We analysed the samples within six hours by gas chromatography using a Porapak N column and flame ionization detector for CH₄, and Porapak Q and electron capture detector¹³ for N₂O from the same syringe. Flux measurements were made mid-morning on each sampling day, generally weekly at each site. We tested sampling time periods ranging from 10 to 60 minutes and taken at 2 or 3 time intervals, and in all cases CH₄ uptake was greater during the first sampling period than in subsequent periods. We therefore calculated CH₄ uptake rates assuming first-order kinetics rather than linear decay as had been suggested⁶. Measurements of CH₄ and N₂O fluxes were made at 6:00, 12:00 and 18:00 on several days. Over a 24-hour period, changes in CH₄ uptake averaged 10% or less of the mean, whereas the spatial and temporal variation in N₂O emissions was much higher¹⁴.

We collected soil samples (0–15 cm) at the time of each gas flux measurement. The soils were analysed gravimetrically for soil water content and extracted with 2M KCl, and the extracts analysed colorimetrically for ammonium and nitrate content. We analysed soils from each site for total carbon and nitrogen content with a combustion C/N analyser. From July, we measured the soil temperature at a depth of 2.5 cm at each site to supplement the continuous soil temperature readings being made near the catena.

TABLE 1 Physical and chemical properties of soils and flux rates of CH₄ and N₂O from each site

Site	pH	Soil analysis*		NO ₃ ⁻ mg N per kg	NH ₄ ⁺ mg N per kg	n	Gas flux rates§		n	N ₂ O emission	
		Total N (%)	Total C				CH ₄ uptake $\bar{X}$ (g C ha ⁻¹ d ⁻¹)	s.d.		$\bar{X}$ (g C ha ⁻¹ d ⁻¹)	s.d.
Swale†											
Fertilized	5.6	0.223	2.215	5.1	0.7	128	3.6	1.6	108	6.2	15.5
Unfertilized	6.0	0.189	1.841	1.6	0.9	136	3.6	1.3	116	3.0	3.7
Midslope‡											
Fertilized	5.6	0.138	1.216	1.3	0.9	124	4.1	1.4	112	3.1	4.0
Unfertilized	6.5	0.141	1.245	1.1	0.9	132	6.3	2.1	120	1.8	1.7
Pasture‡											
Fertilized	5.6	0.138	1.177	6.4	20.3	180	3.8	1.8	162	6.1	10.7
Unfertilized	6.2	0.120	1.066	1.0	1.5	180	5.8	1.9	162	2.5	2.4
Wheat‡	5.8	0.116	0.955	1.1	1.4	162	1.3	0.6	162	2.6	2.7
Fallow‡	6.0	0.111	0.850	10.1	1.9	162	1.8	0.8	162	4.5	6.7
Grass‡	6.4	0.104	0.841	0.6	1.3	162	2.6	1.1	162	3.5	5.6

* Data are means for soil samples (0–15 cm) collected on each gas sampling day.

† The swale is a sandy clay loam soil classified as a Pachic argiustoll.

‡ These are sandy loam soils classified as Ustollic haplargids.

§ n , number of samples; $\bar{X}$, mean; s.d., standard deviation.

Methane uptake rates in the unfertilized grassland averaged 3.6, 5.8 and 6.3 g carbon per hectare day (g C ha⁻¹ d⁻¹) in the swale, CAFS and midslope sites, respectively, between March and December 1990 (Fig. 1 and Table 1). Methane uptake at the swale was significantly less ($P < 0.01$) than at the CAFS and midslope sites. The midslope and CAFS soils were similar in texture and N content, whereas the swale site had finer texture and higher total N content (Table 1). The soil nitrate and ammonium contents of the three unfertilized soils were similar ($P > 0.05$). Annual *in situ* N mineralization at the midslope is, however, substantially less than in the swale¹⁰ (41 compared with 55 kg N ha⁻¹ yr⁻¹), suggesting that it is the N turnover (mineralization and nitrification)¹⁵, rather than the mineral N content, which directly influences the CH₄ uptake.

Nitrous oxide efflux from the soils was inversely related to methane uptake (Fig. 1), averaging 3.0 and 1.8 g N ha⁻¹ d⁻¹ in unfertilized swale and midslope sites, respectively, compared to 3.6 and 6.3 g C ha⁻¹ d⁻¹. The nitrous oxide flux from all sites was highest immediately following precipitation, when CH₄ uptake was lowest. If the fluxes of N₂O (ref. 11) and CH₄ (ref. 16) were both largely mediated by ammonium-oxidizing bac-

teria, then CH₄ uptake may have begun when the soil moisture declined to the point where slowed ammonium diffusion limited nitrification, although our data do not preclude other explanations. Under very dry conditions, both CH₄ uptake and N₂O emissions declined, and they remained low until soil water content increased.

Nitrogen fertilization decreased CH₄ uptake (Fig. 1), as was found for temperate forest soils⁶. In the AFS plots, annual N applications caused the CH₄ uptake to decrease by an average of 41% ($P < 0.01$). Methane uptake by the midslope site decreased from 6.3 to 4.1 g C ha⁻¹ d⁻¹ (Fig. 1) as a result of applying 45 g m⁻² of urea-N in 1981 ($P < 0.01$). A similar N application to the more fertile swale soil in 1982 did not alter CH₄ uptake, which averaged 3.6 g C ha⁻¹ d⁻¹. These data suggest that high N turnover, whether native or due to fertilization, suppresses CH₄ uptake.

Nitrogen fertilization increased N₂O emissions by a factor of 2–3 at all sites ($P < 0.05$), as had been found earlier¹¹. Overall, N₂O emissions from the midslope and swale sites were 2–4 times greater in 1990 than in 1981 or 1982 (ref. 11), possibly due to wetter conditions in 1990. The inverse relationship between N₂O

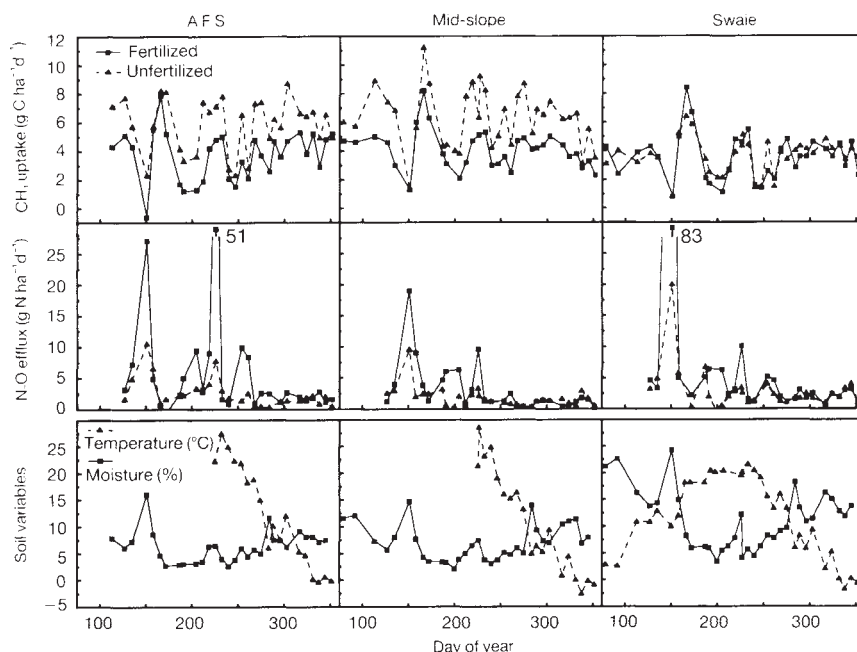


FIG. 1 Effect of landscape position and nitrogen-fertilization on CH₄ uptake and N₂O emission in shortgrass prairie soils. Soil temperatures and water contents were measured in unfertilized soils.

flux and CH_4 uptake was apparent with or without nitrogen fertilization. The influence of nitrogen fertilization on N_2O flux was most evident in moist soils, whereas its influence on CH_4 uptake was most evident as soils began to dry after rain (Fig. 1).

To determine whether cultivation affects CH_4 uptake in grassland soils, as observed in tropical soils⁷, we began monitoring CH_4 uptake in the wheat-fallow system. During 6 months of weekly measurements, CH_4 uptake averaged 2.6, 1.8 and $1.3 \text{ g C ha}^{-1} \text{ d}^{-1}$ in native grassland, fallow and wheat sites, respectively (Fig. 2). These data indicate that disturbing the natural grassland decreased CH_4 uptake ($P < 0.01$). Soil moisture contents averaged 8.6, 11.1 and 12.9% in grassland, fallow and wheat, respectively. Soil ammonium content did not differ among the three sites but soil nitrate was on average ~10 times higher in the fallow soil than in the other two sites. Long-term experimentation and frequent flux measurements are needed to understand the reasons for differing CH_4 uptake rates in different soils and crop pastures.

Our data indicate that the semi-arid grasslands represent a significant global sink for CH_4 (ref. 8). Methane uptake in the grassland ranged from 6 to $61 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, compared with uptake rates of 6–24, 52, 0–112, and 10–160 $\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ in tropical forest¹⁷, subtropical broad-leaved savannah¹⁸, tundra¹⁹ and temperate forest soils^{6,8}, respectively. If our measurements are representative of CH_4 uptake by these grasslands globally, then 0.5 to 5.6 Tg of CH_4 are removed from the atmosphere in these grasslands each year.

Our data suggest that nitrogen fertilization in natural ecosystems increases N_2O emissions and decreases CH_4 uptake⁶. These

data also indicate that recent changes in use or management of grasslands, such as cultivation, have decreased CH_4 uptake from and increased N_2O efflux to the atmosphere. In addition to increased production from rice paddies, ruminants and other sources²⁰, decreased consumption by soils may contribute to increasing atmospheric methane. □

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Phases and electrical conductivity of a hydrous silicate assemblage at lower-mantle conditions

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THE presence of a small amount of water in the lower mantle might affect in a significant way the geophysical and geochemical properties of its host mineral assemblage^{1–5}. Here we present experimental observations of the phase behaviour and the electrical conductivity of a hydrous silicate assemblage synthesized from a mixture of $(\text{Mg}_{0.88}\text{Fe}_{0.12})\text{SiO}_3$ pyroxene and water under the pressure and temperature conditions of the lower mantle. Previous studies have shown that anhydrous $(\text{Mg}, \text{Fe})\text{SiO}_3$ pyroxene transforms to a perovskite structure under these conditions^{6–9}. We find that, although the hydrous assemblage is also dominated by the $(\text{Mg}, \text{Fe})\text{SiO}_3$ perovskite phase, it coexists with the so-called hydrous phase D, of estimated composition $(\text{Mg}, \text{Fe})\text{SiH}_2\text{O}_4$. Our measurements show that the inclusion of small amounts of water in the silicates can enhance the electrical conductivity of the lower-mantle assemblage by more than three orders of magnitude at these temperatures and pressures.

Our starting material was prepared by homogeneously mixing a $(\text{Mg}_{0.88}\text{Fe}_{0.12})\text{SiO}_3$ pyroxene with $4(\pm 2)\%$ by weight of distilled water, giving a $(\text{Mg}_{0.88}\text{Fe}_{0.12})\text{SiO}_3/\text{H}_2\text{O}$ molar ratio of 4/1. The pyroxene was a natural enstatite from Bamble, Norway, which was ground to a grain size $\leq 1 \mu\text{m}$ before mixing. The high-pressure perovskite phase synthesized from anhydrous Bamble enstatite has been carefully documented in previous studies (for example, refs 4, 7, 10).

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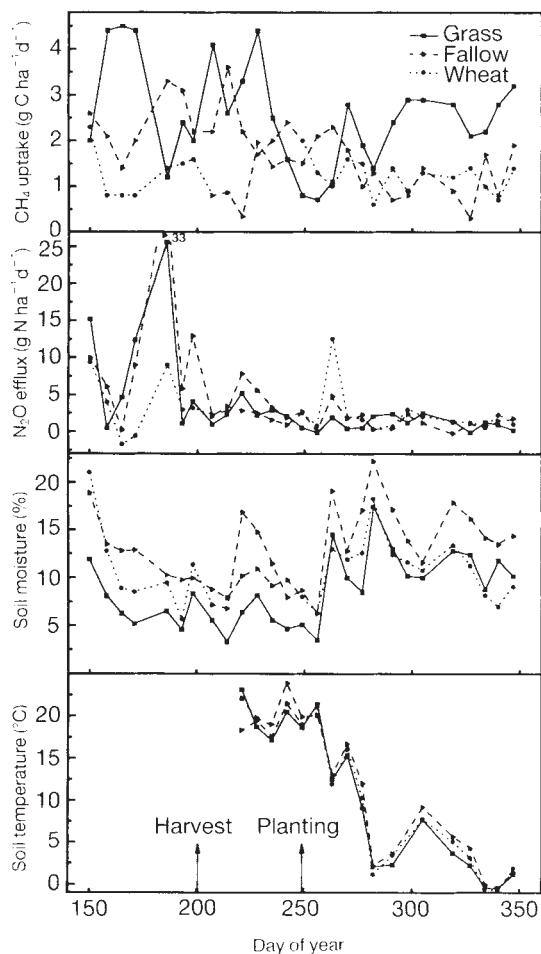


FIG. 2 Effects of wheat cropping on CH_4 uptake and N_2O emissions in shortgrass prairie soils. Arrows indicate approximate dates of harvest and planting, when the wheat and fallow designations switch.

Our experiments were performed with a Mao-Bell-type diamond-anvil cell and a continuous-wave Nd:YAG laser-heating system. To avoid chemical contamination, no pressure medium or ruby (for pressure measurement) was added to the sample. Instead, pressure was determined by calibrating the spring length of the diamond cell using the ruby-fluorescence scale⁴, giving uncertainties in pressure of about ± 5 GPa. To seal the sample between the diamond anvils, we used a 'punched-out' gasket, but to avoid reaction with the stainless-steel gasket material, the outer edge of the sample was not heated^{4,10}. The average temperatures of the sample during laser heating were determined by analysing the thermal-radiation (black-body-like) spectrum of the sample, with temperature uncertainties of 50–300 K at temperatures of over 1,000 K; uncertainties were less than 5 K at ambient temperature. The experimental techniques have been described in detail elsewhere^{4,10}.

The starting mixture was first compressed in the cell to pressures of ~ 40 –60 GPa, and then converted to the high-pressure assemblage by laser-heating to temperatures of $\sim 2,000$ K. The occurrence of phase transition was confirmed by visual observation of the sample colour using transmitted light: the colour changes from light yellow to brownish as the starting phases transform to the high-pressure phase assemblage.

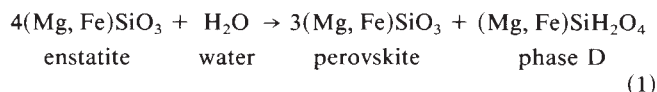
The resulting high-pressure assemblage was then analysed by X-ray diffraction, after quenching the sample to ambient conditions and removing the unheated portion. We have observed 19 diffraction lines from this assemblage, using filtered Cu K α radiation with the Debye-Scherrer technique. The interplanar d spacings of these lines, along with their intensities, are listed in Table 1. Among these lines, 14, including the strongest, can be assigned to the perovskite phase and are well fitted using the lattice parameters $a = 4.789$ Å, $b = 4.926$ Å and $c = 6.902$ Å (see Table 1). These lattice parameters are in full agreement with those of perovskite synthesized from anhydrous Bamble enstatite^{7,8}. Therefore we conclude that perovskite is also the dominant phase of the hydrous iron-magnesium silicate assemblage at lower-mantle conditions. From our lattice-parameter determinations, we see no change in the Fe/Mg ratio of the perovskite relative to the starting pyroxene composition.

In addition to those of the perovskite phase, two extra diffraction lines are clearly observed, which we assign to phase D, a high-pressure hydrous silicate phase which was first found by Liu^{2,3} in studies of serpentine at pressures between 22 and 28 GPa (see Table 1). Liu³ inferred that phase D has the composition (Mg, Fe)SiH₂O₄.

We confirmed the presence of extra diffraction lines, beyond those of the perovskite phase alone, by direct comparison with X-ray patterns obtained from anhydrous Bamble enstatite that had been laser-heated at pressures of ~ 25 –125 GPa (refs 4, 7, 10; compare ref. 19). Specifically, we observed both the strongest diffraction line of phase D ($d \approx 3$ Å), and the characteristic diffraction line of phase D at low scattering angles corresponding to $d \approx 10$ Å (see Table 1 and ref. 3). Because the phase-D lines are relatively weak, and the diffraction pattern from our sample is complex, we give large uncertainties to the observed d -spacings (Table 1). In addition, the remaining six published³ diffraction lines for phase D are either weakly present or overlap with perovskite lines and might therefore be obscured in our patterns. Finally, the weak line with $d = 7.6$ (± 0.7) Å cannot be indexed with the perovskite structure (see Table 1), yet because its d value is consistent with the phase-D group, we assign it to this phase even though it has not been reported by Liu.

At pressures above 22 GPa, Liu^{2,3} has observed a mixture of periclase plus phase D after heating serpentine samples to about 1,300 K. From these phases, Liu³ proposed that hydrous enstatite would transform to a phase assemblage of perovskite, periclase (or magnesio-wüstite for iron-bearing samples) and phase D at pressures over 22 GPa. Our present results are, in general, consistent with Liu's prediction, except that we observed no periclase or magnesio-wüstite. Considering the stoichiometry of

the starting enstatite+water mixture, our X-ray diffraction results suggest that, at lower-mantle conditions, the following reaction takes place:



In other words, our results support the compositional formula of phase D proposed by Liu³. Moreover, our experimental results imply that periclase is not a separate phase at lower-mantle conditions in the hydrous silicate system with a MgO/SiO₂ mole ratio exactly equal to 1—that is when the silicate contained in the system has pyroxene composition. The periclase phase observed by Liu³ in the high-pressure hydrous assemblage therefore probably results from a MgO/SiO₂ ratio larger than unity in his starting serpentine.

Based on the stoichiometry of our starting material and the weak intensities of the observed diffraction lines, we estimate that our assemblage contains $\leq 25\%$ by weight of phase D, with the perovskite phase comprising ≥ 75 wt% of the hydrous assemblage. The fact that the perovskite composition is apparently identical to that of the initial pyroxene indicates that there is no preferential partitioning of Fe or Mg between perovskite and D phases. Therefore, we infer that the Fe/Mg ratio of phase D is the same as that of the perovskite.

To measure electrical conductivity, the samples were loaded in the cell along with two platinum leads. Again, a 'punched-out' gasket was used to seal the sample inside the cell, although the gasket was now coated with an electrically insulating layer^{4,10}. The electrical conductivity was measured both before and after the samples had been converted to the perovskite+phase-D (Pv+D) assemblage at pressures between 40 and 55 GPa and at temperatures of 1,800–2,000 K. The conductance across our sample increased markedly on conversion of the hydrous pyroxene to the Pv+D assemblage: for example, on conversion at 55 GPa, the electrical current measured at 6.02 V and a frequency of 899 Hz increased from 20.2 (± 0.1) nA to 418.5 (± 0.3) nA.

TABLE 1 X-ray diffraction data for the high-pressure phases of the hydrous silicate enstatite

hkl	Experimental data*		hkl	Calculated Pv phase†		hkl	Observed phase D‡	
	d (Å)	Phase		d (Å)	I/I_0		d (Å)	I/I_0
30	1.392 (± 0.003)	Pv + D	312	1.390	20	20	1.390	
20	1.418 (± 0.003)	Pv	132	1.416				
6	1.480 (± 0.003)	Pv (+D?)	311	1.483	20	1.496		
10	1.517 (± 0.003)	Pv	131	1.515				
3	1.61 (± 0.06)	D			80	1.61		
6	1.677 (± 0.005)	Pv	023	1.681				
50	1.722 (± 0.006)	Pv	004	1.726				
8	1.855 (± 0.006)	Pv	122	1.849				
8	1.913 (± 0.004)	Pv	113	1.911				
6	1.973 (± 0.006)	Pv	202	1.967				
10	2.010 (± 0.006)	Pv	022	2.005				
25	2.070 (± 0.005)	Pv	103	2.074				
10	2.147 (± 0.008)	Pv	210	2.154				
100	2.435 (± 0.015)	Pv (+D?)	112	2.434	15	2.53		
10	2.958 (± 0.086)	D			100	3.00		
10	3.447 (± 0.086)	Pv + D	002	3.451	15	3.37		
1	4.04 (± 0.6)	D			15	4.3		
1	7.6 (± 0.7)	D?						
2	10.3 (± 0.9)	D			10	9.5		

* Observed in this study for the high-pressure assemblage synthesized from the hydrous enstatite. The relative intensities are estimated visually.

† Calculated using the perovskite-type lattice parameters $a = 4.789$ Å, $b = 4.926$ Å and $c = 6.902$ Å.

‡ Reported by Liu³.

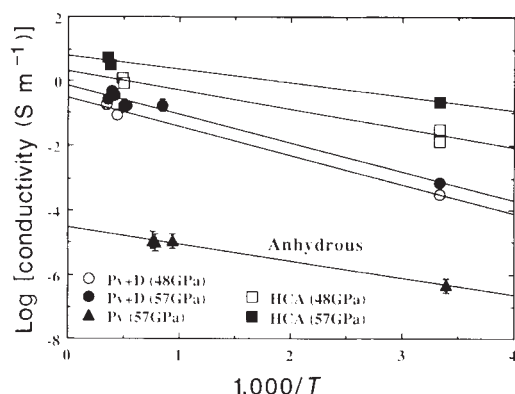


FIG. 1 Logarithm of the electrical conductivity of the perovskite and phase-D assemblage (Pv + D) as a function of $1,000/T$ at 48 GPa (open circles) and 57 GPa (solid circles). The results shown are from two different samples, in which the Pv + D assemblage is synthesized from a mixture of $(\text{Mg}_{0.88}\text{Fe}_{0.12})\text{SiO}_3$ pyroxene with 4 wt% water. The reproducibility of X-ray diffraction and electrical-conductivity measurements for several independent samples, as partially illustrated here, implies that the compositions and conditions of synthesis are reproducible for these hydrous assemblages. The electrical conductivity of the anhydrous $(\text{Mg}_{0.88}\text{Fe}_{0.12})\text{SiO}_3$ perovskite (ref. 10) is also shown (solid triangles). The square symbols represent the experimental results for the high-conductivity assemblage (HCA) obtained by heating the Pv + D mixture at 2,600–2,900 K for over 10 min; here open and solid squares are the electrical conductivities measured at 48 and 57 GPa respectively.

The configuration of the sample chamber and the detailed alternating-current techniques for conductivity measurement at simultaneous high pressures and temperatures have been described in our previous papers¹⁰, which document the uniformity of the temperature across the laser-heated region between the leads. In particular, the sample-lead interfaces achieve temperatures close to the average value in the heated zone because of the efficient absorption of the laser beam and heat transfer by the platinum. Also, we have shown that the laser-heating technique yields activation energies for electrical conductivity that are in quantitative agreement with independent measurements using external heating¹⁴.

The experimental results for the electrical conductivity of the Pv + D assemblage are shown in Fig. 1. For comparison, the conductivity of the anhydrous $(\text{Mg}_{0.88}\text{Fe}_{0.12})\text{SiO}_3$ perovskite measured in our previous studies¹⁰ is also shown in this figure. The electrical conductivity of the hydrous Pv + D assemblage is about three to four orders of magnitude higher than that of the anhydrous perovskite phase. This result is consistent with our previous findings⁴, and demonstrates that the presence of H_2O can greatly enhance the bulk electrical conductivity of the silicate assemblage at lower-mantle conditions.

From these measurements, we calculate an activation energy for electrical conduction in the Pv + D assemblage of 0.19 (± 0.04) eV, which is larger than that found in the anhydrous perovskite (~ 0.11 eV at 57 GPa; see Fig. 1 and ref. 10). Thus the dominant conduction mechanism in this hydrous phase assemblage may be different from that in the pure perovskite. Because of our poor knowledge of the crystal structure and electronic energy-band configuration of phase D, however, the electrical-conduction mechanisms in the hydrous silicate assemblage are not yet clear. We can say only that both intrinsic and extrinsic electrons (electrons excited from the valence band and electrons excited from impurity or defect levels, respectively), as well as protons introduced into the assemblage as water, are candidate charge carriers in the hydrous assemblage.

After heating the Pv + D assemblage at about 2,600–2,900 K for over 10 min and quenching it back to ambient temperature, we found that the colour of the heated sample region changed to black; and tiny glass blobs (a few microns in diameter) were observed in the quenched sample, implying that the product was partially molten at high temperatures. Our measurements show that this black product is one to two orders of magnitude more conductive than the starting Pv + D mixture. The electrical conductivity of this highly conductive assemblage is also plotted as a function of $1,000/T$ in Fig. 1.

To investigate what causes the dark colour and the high conductivity in this highly conductive assemblage, we prepared samples in which the electrical leads were separated by over 30 μm ; that is, the separation between the two platinum wires was larger than the diameter of the laser beam, so that we could focus the laser beam on the sample alone (for details of the

sample configuration, see ref. 10). Hence, unlike the experiments described above, only the sample was heated, the platinum leads remaining cold during laser-heating. In such conditions, we still observed the colour change and the conductivity increase where the sample is heated. Thus, we can rule out chemical reaction between the sample and platinum leads as being the cause of these changes.

Previous studies^{11–13} have shown that a number of electronic-transition processes involving Fe^{3+} ions can produce optical absorption at visible wavelengths in iron-bearing silicates: processes such as intervalence charge transfer, for example, are major causes of light-absorption and colouration in many minerals. For this reason, we interpret the dark colour of the highly conductive assemblage observed in our experiments to be the result of oxidation of a fraction of the iron ions, perhaps to the ferric state. This interpretation is also consistent with the high conductivity, because each Fe^{3+} ion can act as an electron donor to the conduction band and may also provide a path for electronic-hopping conduction^{14–16}. The electronic-hopping model is a possible conduction mechanism in the amorphous phase that we observe, and also cannot be excluded as being an important conduction mechanism in phase D.

Our experimental results suggest that phase D may, at least near the top of the lower mantle, act as the main water-storage phase in the Earth's deep interior. In addition, our measurements have shown that a small amount of water included in the silicate can greatly enhance the electrical conductivity of the assemblage under conditions of high pressure and temperature. Our results may also explain the discrepancy among data reported by different laboratories for the electrical conductivity of silicate perovskite^{4,10,17} (for a detailed discussion, see ref. 18). □

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Streamside trees that do not use stream water

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A LONG-STANDING axiom is that plant distribution is strongly influenced by soil moisture content^{1,2}. While it has been shown that plant taxa inhabiting streamside communities receive or use more water³, it is assumed that this water is obtained from the stream adjacent to where they are found growing. Here we show, using hydrogen isotope ratio analyses at natural abundance levels, that mature streamside trees growing in or directly next to a perennial stream used little or none of the surface stream water. The deuterium to hydrogen content of both source and xylem waters indicated that mature trees were using waters from deeper strata. Although adult trees may have roots distributed continuously throughout a soil profile, it seemed that the most active sites of water absorption were limited to deeper soil layers. In contrast, small streamside individuals appeared to use stream water, whereas small non-streamside individuals used recent precipitation as their primary water source. Our analyses provide both a relatively non-destructive method for assessing water sources of plants and a means of assessing potential competitive interactions among co-occurring taxa. In addition, the method may aid in resolving the role of water in determining plant distributions in areas characterized by sharp soil moisture gradients.

The use of stable isotopes at natural abundance levels in ecological, physiological and environmental research has greatly increased over the past decade⁴⁻⁶. Whereas previous ecological work has emphasized the usefulness of carbon, nitrogen and sulphur isotopes, hydrogen and/or oxygen isotope analyses have proved to be useful for understanding the dynamics of water in atmospheric and geochemical studies^{6,7}. There is no fractionation of isotopes by plant roots during water uptake⁸⁻¹⁰. Therefore, hydrogen isotope analysis of xylem sap, which has not been exposed to evaporative enrichment, should reflect the water sources in use by that plant. Such analyses have made it possible to distinguish quantitatively between recent precipitation and groundwater as water sources for white pines¹⁰ and between fresh- and salt-waters as the water sources for mangrove species¹¹. In both studies, the hydrogen isotope ratio of the possible water sources was significantly different and the percentage of water used from either source was determined by interpolation between the two end members.

In temperate, continental zones, the isotopic composition of precipitation varies both seasonally and with elevation^{12,13}. Precipitation falling in the summer months as rain has a higher deuterium content than winter snows; precipitation falling at higher elevations is depleted in deuterium relative to that at lower elevations^{7,12}. The deuterium to hydrogen content of groundwaters and soil water in deeper layers not exposed to evapotranspiration reflect a weighted average of annual precipitation inputs. However, fossil groundwaters reflect the precipitation inputs of earlier time periods^{6,7}.

In the Intermountain West of the United States, most of the annual precipitation comes in winter as snow, especially at higher elevations^{14,15}. Soil moisture is recharged by this winter precipitation, but then declines quickly to low values by late spring¹⁵. Snow melt from higher elevations supports perennial streams and the development of an extensive streamside riparian vegetation in an otherwise semi-arid region. Although infrequent summer convection storms can occur, they contribute little to

the annual water balance of perennials in these regions¹⁵. Along the Wasatch Mountains of Utah, a scrub oak-maple (*Quercus-Acer*) vegetation type surrounds the streamside (riparian) zone. Both the streamside and adjacent scrub oak-maple vegetation are active in the spring and summer but it is not clear which water sources these plants have available to support metabolic activity during the driest summer months (July–September). Based on precipitation patterns, we hypothesized that streamside trees would use surface stream water while non-streamside plants would be limited to recent precipitation as their primary water sources. Therefore, we examined dominant streamside and non-streamside tree species in the Red Butte Canyon Research Natural Area in the Wasatch Mountains immediately east of Salt Lake City (40° 46' N, 111° 55' E, 1,820–1,890 m elevation). Streamside trees were growing in or within 2 m of a perennial stream. Non-streamside trees were growing 25–45 m from and 2–6.5 m above the stream.

Hydrogen in water was reduced to its diatomic form using zinc¹⁶ and the deuterium content measured on a Finnigan MAT, model delta E, isotope ratio mass spectrometer early in the study, and on a delta S later on. Deuterium content is expressed in delta notation (δD in ‰) relative to the Standard Mean Ocean Water standard¹⁷

$$\delta D = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1,000 \text{‰}$$

where R_{sample} and R_{standard} represent the molar deuterium/hydrogen ratios of sample and standard, respectively. Overall precision of the preparation and analysis is $\pm 1.4\text{‰}$ for the delta E and $\pm 1.0\text{‰}$ for the delta S.

To distinguish between two possible water sources, differences in δD must exist between summer precipitation and stream water. Over a two-year period, significant differences were maintained between stream water and precipitation δD values (Fig. 1). As expected^{12,13}, stream water, derived primarily from snow melt at higher elevations, was lighter (more depleted in deuterium) than summer precipitation. Stream water δD values remained essentially constant at $-121.4 \pm 0.7\text{‰}$ (mean and standard error) over the entire sampling period, whereas individual precipitation events varied between -11‰ in summer and -213‰ in winter, respectively. Over the growing season, soil water adjacent to the stream (-122.1‰) was indistinguishable from that of the stream water (Student's t -test, $t = 0.51$, d.f. = 10, $P = 0.621$), demonstrating that no evaporative enrichment occurred as water may have diffused from the stream through

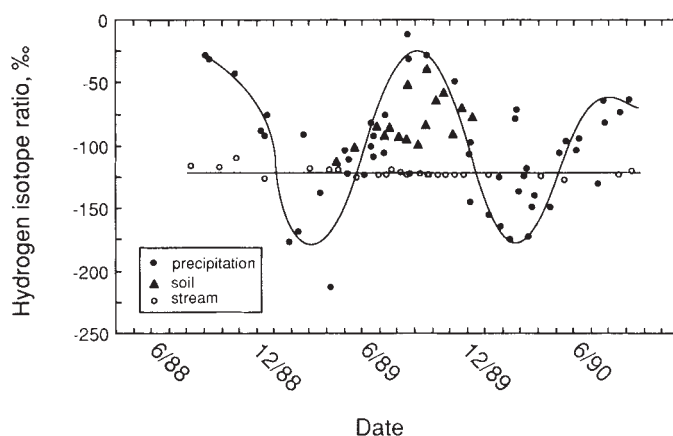


FIG. 1 The hydrogen isotope ratio (δD in ‰) of precipitation, surface stream water and soil moisture at a non-streamside location in the Red Butte Canyon Research Natural Area between July 1988 and September 1990. Soil water δD was obtained by a cryogenic vacuum-distillation extraction from cores collected in the field to a depth of 50 cm. The upper 10 cm of soil was not included to avoid biasing the δD values as a result of evaporative enrichment.

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the soil. In contrast, soil moisture at non-streamside locations had substantially higher δD values, reflecting both spring and summer precipitation input (Fig. 1).

The most common tree species at both streamside and non-streamside sites (*Acer grandidentatum* Nutt., *A. negundo* L. and *Quercus gambelii* Nutt.) were sampled during the peak of the growing season in July. Xylem sap was obtained by cryogenic vacuum distillation of only mature, suberized stem tissues. Younger, partially suberized tissues were isotopically enriched (10–55‰) owing to cuticular water loss and so were not used. Figure 2 shows that the δD values of xylem sap differed among trees, depending both on tree size (based on diameter at breast height; DBH) and location. For all species, δD values of streamside trees with a diameter of less than 20 cm ($-125.5 \pm 9.2\%$) were significantly lighter (Student's t -test, $t = 3.54$, d.f. = 31, $P = 0.001$) than equivalent-sized trees at non-streamside locations ($-89.6 \pm 4.3\%$). Larger individuals, regardless of the site, showed no significant difference in xylem water δD values (streamside = $-133.3 \pm 0.8\%$, non-streamside = $-132.8 \pm 0.9\%$, $t = 0.47$, d.f. = 44, $P = 0.643$).

As anticipated from Figs 1 and 2, small and large trees had significantly different δD values at non-streamside ($t = 7.37$, d.f. = 46, $P = 0.000$) sites. Unexpectedly, different sized trees at the streamside sites also had significantly different δD values ($t = 4.78$, d.f. = 31, $P = 0.000$). Smaller-diameter streamside trees (δD values (-125.5%) were similar to that of the adjacent stream (-123% for this sample date). In contrast, larger streamside trees gave δD values significantly lighter than that of the stream ($t = -13.13$, d.f. = 25, $P = 0.0001$). Thus, whereas young streamside trees were using stream water (because they exhibited equivalent δD values), larger mature trees were not using this same water source. Instead, they either used a lighter water source (more negative δD) or there was isotopic fractionation during water uptake.

We tested for isotopic fractionation during water uptake through roots by growing *Acer negundo* in pots and watering with a known water source. The δD for the water source was $-120.5 \pm 0.5\%$. The δD from suberized stems of ten individually grown trees was $-120.7 \pm 0.5\%$, which is not significantly different from the source water value. This result confirmed

TABLE 1 Hydrogen isotope ratios (δD in ‰) from xylem sap of mature tree species

Species	δD
<i>Betula occidentalis</i> Hook	-129.8 ± 3.2 (5)
<i>Populus angustifolia</i> James	-131.0 ± 3.0 (2)
<i>Prunus virginiana</i> L.	-132.3 ± 4.1 (3)

Data from tree species not shown in Fig. 2. Data are given as means and standard deviation, with the sample size in parentheses. For well water δD was $-132.3 \pm 2.6\%$. The trees stood in or next to Red Butte Creek in the Red Butte Canyon Research Natural Area, Utah.

earlier studies indicating that there was no isotopic fractionation during water uptake^{8–10}, and suggests that larger trees in the field are using a non-surface stream water source.

The most likely alternative water sources are subsurface flows below the stream, coming from higher elevations, or possibly deeper bedrock layers with waters derived from higher elevations. We sampled two springs and one well less than 2.5 km from our study site and an additional well near the head of the drainage basin; the mean δD value of these deeper waters was $-130.7 \pm 1.8\%$. For another two springs and a well within 15 km of the study site, the mean δD for these deeper water layers was $-132 \pm 1.1\%$. The subsurface water samples were collected during three different months and in two different years, suggesting that δD of subsurface water remains relatively stable over the course of time. All subsurface water δD values were similar to that of the largest streamside trees, strongly suggesting that mature trees utilize waters from deeper bedrock layers. We sampled a limited number of mature individuals (> 50 cm DBH) of other common riparian zone trees at the streamside site (Table 2). As before, for each of these species, the xylem sap δD values suggested that the primary source of water was deeper soil layers and not stream water.

Several important ecological implications arise from these results. First, during establishment, these tree species depend on waters in the upper soil layers. Because the isotopic signatures of summer precipitation (non-streamside sites) and surface stream waters (streamside sites) are different, we can distinguish between these two water sources. Second, once established, trees from streamside sites as well as from adjacent non-streamside sites use a deeper and possibly more constant water source, abandoning the water sources on which they were dependent during establishment. By no longer using upper-soil-layer water sources, these species may be able to avoid interspecific competition with more shallow-rooted shrub and herb species that inhabit the same sites. This might ensure a greater probability of survival during droughts common to these semi-arid regions. Studies of plant interaction and competition for water should include the possibility that some trees might use deeper water sources. Such a conclusion may not be evident when looking at comparative leaf water potential values, but requires knowledge of the exact water source used by the plant, which can best be obtained through hydrogen isotope analysis. □

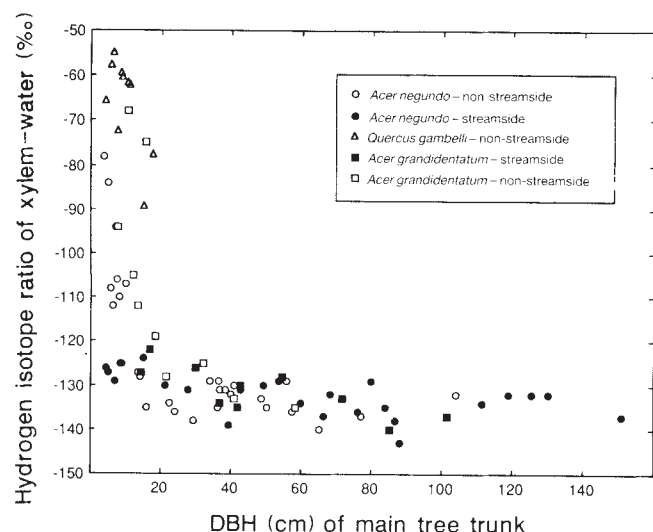


FIG. 2 The hydrogen isotope ratio (δD in ‰) obtained from xylem sap of three common streamside (closed symbols) and adjacent non-streamside (open symbols) tree species in the Red Butte Canyon Research Natural Area in 1989 as a function of the diameter at breast height (DBH) of the main trunk. Mean δD values of stream water (from Fig. 1) and local well water (see text) were $-121.4 \pm 0.7\%$ and $-132.3 \pm 2.6\%$ respectively. Methods are outlined in the text and in ref. 10.

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Plumage coloration is a sexually selected indicator of male quality

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FEMALE choice of mates based on the expression of characters that correlate with male quality remains a controversial and largely untested idea¹. By choosing quality males, females stand to gain resources², genetic benefits for their offspring^{3–5}, or both. In the house finch (*Carpodacus mexicanus*), male plumage coloration is a function of dietary intake of carotenoids^{6,7}. Here I present results of field studies that indicate that females prefer to mate with colourful males and that plumage brightness correlates with a male's capacity for parental care and perhaps its genotypic quality. Artificially brightened males paired more quickly and frequently than sham control or lightened males. Among unmanipulated males, plumage coloration was correlated with nest attentiveness and overwinter survival. In addition, there was a positive correlation between the coloration of fathers and sons.

The house finch is a small, sexually dichromatic passerine that is socially monogamous^{8,9}. Unlike many passerine species, house finches do not defend territories during the breeding season^{9,10}, although both males and females defend mates from rivals. Pairing begins several weeks before the start of nestbuilding and, once paired, house finches form tight associations.

Paired individuals are rarely seen alone until the female begins to incubate, so the pairing status of individuals can be determined unambiguously before nesting. Within populations, males display continuous variation in plumage coloration, ranging from pale yellow to bright red^{9,11}. Captive feeding experiments indicate that all male house finches have the same potential to display bright or drab plumage⁷ and that variation in plumage coloration reflects the type and quantity of carotenoids ingested by individuals at the time of their annual moult^{6,7}. In laboratory mate-choice experiments with wild-caught house finches, females display a significant preference for the reddest male available⁹.

To determine whether female house finches use male plumage coloration as a criterion for mate choice in the wild and to investigate whether male plumage coloration might be an indicator of male quality, I studied a breeding population of house finches at Ann Arbor, Michigan. Between 10 February and 21 March in 1989 and 1990, I used hair dyes to brighten 40 males and hair lighteners to lighten 40 males. I also assigned 20 males as sham controls by treating them with colour developer which did not alter plumage coloration. There were no significant differences among treatment groups in the size or pre-manipulation plumage scores of males, but all three groups differed significantly in post-manipulation plumage scores (Table 1). The range of plumage scores of manipulated males (96–177) fell within the range of natural plumage coloration in the Ann Arbor population (87–177).

There were no significant differences in dispersal or mortality between manipulated and unmanipulated males ($\chi^2 = 0.12$, d.f. = 1, $P > 0.25$) or among manipulated males used in different treatments (Table 1). Brightened males were significantly more likely to attain a mate than lightened or sham-control males, and sham-control males were more successful than lightened males (Table 1). Of the males that paired, brightened males paired earlier than lightened or sham-control males (Table 1), but the differences were not significant. Thus, field experiments indicate that female house finches prefer to pair with brightly plumaged males.

Male house finches feed their mates during incubation as well as their offspring^{8,12}. To test whether male coloration reflects a male's ability to provide food for its mate and offspring, I recorded the time between feeding visits by males at 32 nests. I found a significant correlation between male attentiveness

TABLE 1 Results of plumage coloration manipulation experiments

Male characteristics	Brightened (N=40) $\bar{x}$ (s.d.)	Sham control (N=20) $\bar{x}$ (s.d.)	Lightened (N=40) $\bar{x}$ (s.d.)	Test statistic	P
Weight (g)	21.9 (1.3)	22.3 (1.4)	21.8 (1.3)	0.87*	0.42
Wing length (mm)	80.2 (1.8)	79.3 (1.6)	80.3 (2.3)	1.83*	0.17
Original plumage score	140.7 (12.5)	139.9 (14.3)	141.0 (11.4)	0.05*	0.95
Manipulated plumage score	161.6 (7.9)	139.9 (14.3)	129.4 (10.3)	98.7*	0.0001
Number resighted (proportion resighted)	23 (0.58)	10 (0.50)	26 (0.65)	1.30†	>0.25
Number paired (proportion paired)	22 (1.00)	6 (0.60)	7 (0.27)	24.75†	0.0001
Time to pair (days)‡	12.1 (12.5)	20.2 (17.8)	27.8 (22.4)	2.82*	0.07

Higher plumage scores correspond to brighter plumage; see ref. 9 for details of scoring technique. I captured house finches at feeding stations and individually marked them with one aluminium and three coloured plastic leg bands. Males used in the experiment had dye, lightener, or colour developer applied to pigmented regions of their plumage⁹ at times between 13:00 and 15:00 on the day they were trapped. After excess colourant was rinsed from their feathers, males were placed in front of a heat lamp for 2 h to dry, held overnight with food and water *ad lib*, and released at 10:00 the next morning. All manipulations were completed before the onset of nestbuilding in the study population. Only previously unbanded males were included in the manipulation. They were assigned to treatment groups so that each group received approximately the same number of males each day and so that males in each treatment were similar in size and pre-manipulation plumage score. Males were counted as 'resighted' if they were seen at least 48 h after release and 'paired' if they were seen in exclusive association with a female. In 1990, one brightened male was trapped 3 weeks after release but was otherwise not seen, so this male was counted as resighted but was excluded from pairing data. There were no significant differences between years in the pre-manipulation plumage scores or sizes of males or in the effects of the manipulations, so data were pooled for 1989 and 1990.

* F, One-way analysis of variance (ANOVA).

† χ^2 , One-tailed test.

‡ Only males that paired were counted in the time to pair comparison, as measured by the interval between release following manipulation and exclusive association with a female.

TABLE 2 Nest and mate desertion by females

	Successful (<i>N</i> = 78)	Failed (<i>N</i> = 60)	Abandoned (<i>N</i> = 17)	Test statistic	<i>P</i>
Number reneesting (proportion reneesting)	42 (0.54)	41 (0.68)	9 (0.53)	3.28†	>0.10
Number reneesting with same male (proportion)	42 (1.00)	38 (0.93)	1 (0.11)*	55.87†	0.001
Mean plumage score (s.d.)	152.3 (8.9)	152.2 (6.5)	144.4 (12.3)	6.02‡	0.003

Success of first nesting attempt by pairs, pooled for 1988–90. Nests were deemed successful if young fledged, failed if part or all of the nest contents disappeared before fledging and abandoned if a complete, intact clutch or brood was deserted.

* The female from the only pair to reneest together after abandoning a nest, abandoned the second nest as well.

† χ^2 , One-tailed test.

‡ *F*, One-way ANOVA.

(mean rate of feeding visits) and male coloration (Fig. 1a), indicating that plumage coloration is a reliable signal of male parental investment. Choosing an inattentive male as a mate seems to impose direct cost on the female. Eleven per cent of females abandoned both their nest and mate before eggs hatched (Table 2); males that were deserted were significantly less

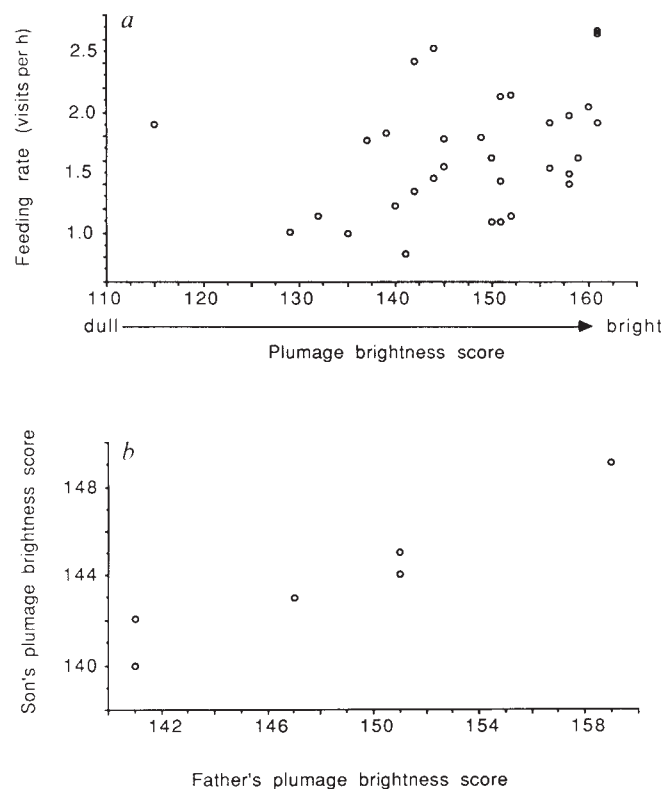


FIG. 1 *a*, Male nest attentiveness (mean rate of feeding visits) in relation to plumage score ($r_s = 0.42$, $n = 32$, $P < 0.05$). Feeding rate was measured by observing the interval between visits by the attending male. Two or three feeding intervals, recorded at least 24 h apart, were obtained for each male and all observations were conducted at times between 06:00 and 13:00 during incubation (between laying of the last egg in a clutch and hatching of the first egg). To test for age effects, I repeated the comparison using only the attendance rates of 13 males known to be two years old or older. Again, there was a significant positive correlation between male plumage score and attentiveness ($r_s = 0.63$, $n = 13$, $P < 0.05$). *b*, Relationship between the plumage score of fathers and sons ($r_s = 0.91$, $n = 6$, $P < 0.01$). Sons were banded in a nest for which the attending male's plumage score was known. The plumage score of sons was recorded the following year when they returned to the study site as yearling males. Of the seven sons that returned, two came from the same nest and the remaining five came from different nests attended by different males. To maintain independence of points, a single mean plumage score value for the brothers was used in the analysis.

colourful on average than males that were not deserted (Table 2). Females depend on their mates for food during incubation and cases of desertion by females probably result from inadequate provisioning by males.

In addition to resource benefits, females may also gain genetic benefits by choosing to mate with a colourful male. I compared the mean coloration of males banded in 1988 or 1989 and which returned in the following year with the mean coloration of males that did not return. To make the comparison less susceptible to dispersal bias, I also considered only males that paired in 1988 or 1989. In all comparisons, returning males were significantly more colourful than males that did not return (Table 3), suggesting that colourful plumage is an indicator of male viability and perhaps genetic quality¹³, although the relationship between colour and viability may be due to phenotypic effects.

In support of the idea that plumage coloration may be used by females to assess male genotypic quality, is a correlation between the coloration of fathers and sons. According to the good genes models of sexual selection, the overall genotypic quality of a male determines how well it will perform in its environment and the degree to which it will achieve maximum expression of a display trait^{14–17}. These models predict a positive correlation between character expression of father and son^{14–17}. I compared the plumage scores of seven males that were banded as nestlings and returned the following year to the plumage scores of their fathers and found a significant positive correlation (Fig. 1b). Although this correlation fits predictions of the 'good genes' models, it could also be due to maternal or paternal effects as colourful males tend to provide more food and probably pair with higher quality females than drab males.

These observations indicate that female house finches prefer brightly plumaged males and that, by choosing a colourful male, females gain resources and possibly genetic benefits for offspring. This result provides empirical support for the idea that plumage coloration serves as a signal of male quality⁴ and corroborates previous studies on intersexual selection for integumentary pigmentation in fish^{18–22}. Carotenoid pigmentation

TABLE 3 Male plumage coloration in relation to overwinter survival

Interval	Status	<i>N</i>	Plumage score $\bar{x}$ (s.d.)	<i>t</i> *	<i>P</i>
1988–89	All returned	63	148.0 (9.1)	1.66	0.05
	All not returned	87	145.2 (10.9)		
	Paired returned	36	150.6 (7.9)	1.70	0.05
	Paired not returned	32	147.3 (7.9)		
1989–90	All returned	82	149.1 (10.8)	2.45	0.007
	All not returned	130	144.7 (13.7)		
	Paired returned	55	152.0 (10.1)	1.89	0.03
	Paired not returned	39	148.1 (9.3)		

Males were banded or sighted between 1 February and 1 July in year 1. Males counted as returned were trapped or sighted between 1 February and 1 July in year 2.

* One-tailed *t*-test comparing mean plumage scores of adjacent rows.

tion may prove to be a display character that has independently evolved as a viability indicator in a diverse array of taxa. □

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The cyclops mutation blocks specification of the floor plate of the zebrafish central nervous system

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THE floor plate is a set of epithelial cells present in the ventral midline of the neural tube in vertebrates¹ that seems to have an important role in the developmental patterning² of central nervous system fibre pathways^{3,4}, and arrangements of specific neurons⁵. The floor plate arises from dorsal ectodermal cells closely associated with the mesoderm that forms notochord⁶, and it may depend on interactions from the notochord for its specification. To learn the nature of these interactions we have analysed mutations in zebrafish (*Brachydanio rerio*). We report here that in wild-type embryos the floor plate develops as a simply organized single cell row, but that its development fails in embryos bearing the newly discovered zygotic lethal 'cyclops' mutation, *cyc-1(b16)*. Mosaic analysis establishes that *cyc-1* blocks floor plate development autonomously and reveals the presence of homeogenetic induction between floor plate cells.

The floor plate (FP) can be visualized during key stages of embryonic development in the spinal cord by the distinctive cuboidal morphology of its cells, and by labelling with the monoclonal antibody zn-5. It is positioned precisely in the midline just beneath the central canal, and is but a single cell wide (Fig. 1). In contrast, FP structure in most vertebrates is more complex. The antibody zn-5 seems to recognize a cell-surface antigen⁷, whose expression by FP cells is strongest on faces apposed to one another, in contrast to facing their lateral central nervous system (CNS) and ventral (notochord) neighbours. The labelling by zn-5 also indicates that the FP extends through the hindbrain and midbrain⁷, and ends abruptly at the midbrain/forebrain boundary. In the one-day embryo FP cells

in the hindbrain are more flattened in the anterior–posterior dimension than are those in the spinal cord, and they contact one another in a staggered linear arrangement rather than in simple end-to-end fashion (Fig. 1a, d).

Early cells that will form the FP lie dorsally, close to notochord precursors, and single clones founded during blastula stages frequently include both cell types⁸. Cellular intercalations⁹ elongate the two primordia into longitudinal rows, the FP overlying the notochord. Cell transplantation (Fig. 1b, c) and clonal

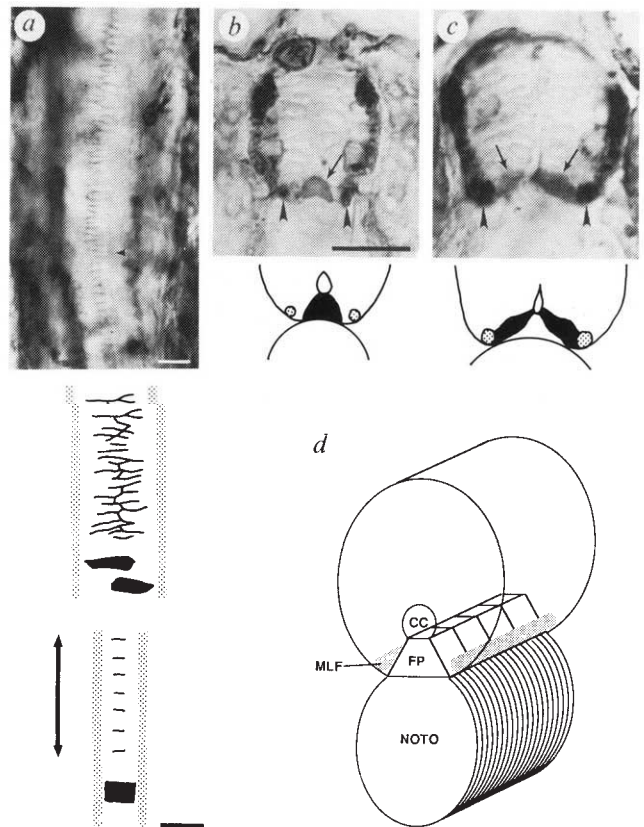


FIG. 1 The zebrafish embryonic FP is simply organized. *a*, The zn-5 monoclonal antibody labels the surfaces between adjacent FP cells. Dorsal view of the rostral spinal cord at 28 h postfertilization at 28.5 °C. Along most of the length of the spinal cord the FP is a single linear cell row (see ref. 4 for an alternative interpretation), and this structure changes at about the fourth spinal segment (arrowhead) to a staggered row, which continues into the hindbrain. The interpretive drawing shows this patterning in the spinal cord (lower) and hindbrain (upper). In both regions the ventral CNS fibre pathways termed the medial longitudinal fascicles (MLF; stippled) flank the FP. The double-headed arrow indicates one segment length. The number of FP cells per segment varies from about 25 in the hindbrain to 3 in the caudal spinal cord. *b*, *c*, Transverse sections of spinal cord show that the MLF (arrowhead; as labelled with the monoclonal antibody zn-12²³) is separated from the FP (arrow; as labelled by injection with lineage tracer in *b*), but contacts its neighbouring cells (arrows in *c*). *d*, Schematic representation of the topological relationships of the FP in the spinal cord. The FP underlies the central canal (CC), and is specifically juxtaposed to the underlying notochord (NOTO), also present as a single row of cells.

METHODS. Whole embryos were labelled by injection of a mixture of 5% rhodamine-dextran and 5% fixable biotin-dextran (Molecular Probes) into the yolk region of the embryo at the 2–8 cell stages²⁴. The tracers spread through the cytoplasm to all of the blastoderm cells. Later, 10–100 cells within or near the embryonic shield of the early gastrula were orthotopically transplanted to unlabelled hosts²⁴. Hosts with labelled ventral CNS cells were selected by inspection of rhodamine fluorescence at 28 h, and the biotin labelling was developed histochemically with streptavidin/horseradish peroxidase (Vectastain) and diaminobenzidine, allowing detailed observation of the morphology of individual cells. The methods for immunohistology were as previously described²³. After whole-mount staining, selected embryos were embedded in plastic and sectioned at 6 µm. Scale bars, 20 µm.

analyses (not shown) suggest that in zebrafish each lineage is a single cell wide. This one-dimensional patterning is unique to these two structures; other cell types that we know of are bilaterally paired.

To identify genes that pattern the development of these and other cell types of the embryonic axis we have systematically screened embryos from γ -ray-mutagenized stocks¹⁰ for morphological axial defects¹¹. The 'cyclops' mutation, *cyc-1(b16)*, was obtained from such a screen, and seems to identify a locus directly involved in specification of the FP. The mutation is transmitted in a mendelian fashion, and produces a severe pleiotropic recessive lethal phenotype, including cyclopia and curving of the body axis (Fig. 2). The fusion of the two lateral eyes into a single median ventral cyclopic one is seemingly due to the absence of ventral forebrain tissue that normally keeps the eyes separate. No prominent cell death occurs throughout at least 24 h; possibly the missing tissue never forms.

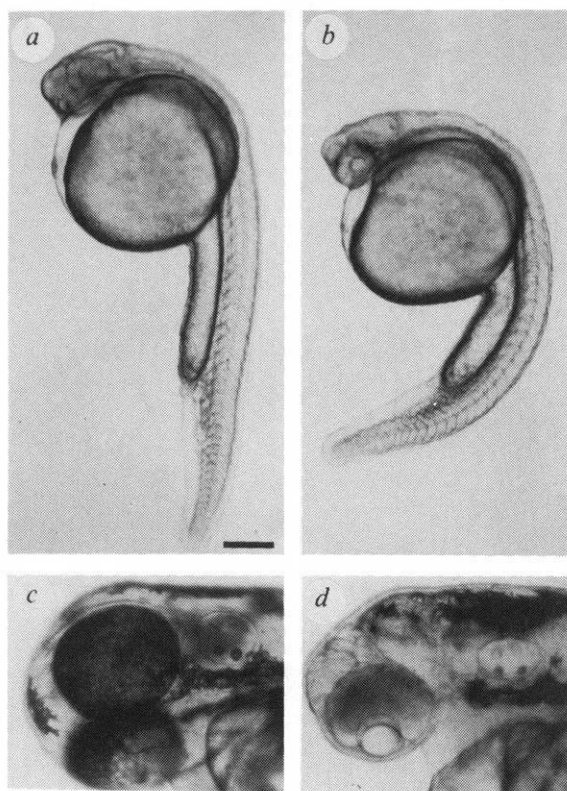


FIG. 2 The *cyc-1(b16)* mutation disrupts forebrain derivatives and curves the body axis. Left side views of living embryos, with wild-type siblings shown to the left (a, c) and homozygous *cyc-1* mutants to the right (b, d). a, b, 26 h showing the ventral curvature of the *cyc-1* body. c, d, Higher magnification of the head at about 72 h, showing the cyclopic eye ventral to the brain. The extent of fusion of the eye primordia is variable among the mutants and in this example is particularly severe; more typically the cyclopia is only partial. Although not visible in these micrographs the olfactory placodes, derived from rostral epidermis¹⁶ and normally paired, are incompletely and variably fused together in the fashion of the fusion of the eyes (T. F. Schilling, personal communication). The *cyc-1* phenotype is zygotic recessive, and mutants survive for less than four days of development. The mutation segregates as a single locus: 23.2% of diploid embryos obtained from crosses between heterozygotes were mutant (691/2976), and 45.9% of haploid embryos obtained from heterozygous females were mutant (201/438). In both experiments the fraction of mutants observed was slightly less than expected (1/4 and 1/2 respectively), which could be due either to incomplete penetrance or to a lethal effect. Half-tetrad analysis yields a gene-centromere distance of 28 centimorgans (1,045 mutants among 4,656 total progenetic diploid progeny obtained from heterozygous females by the 'EP' method²⁵; the calculation of map distance assumes complete crossover interference²⁶). Scale bars, 200 μ m.

Whereas the cyclops phenotype is very severe in CNS regions rostral to the second rhombomere, we found a more subtle change, the depletion of the FP, along the remaining neuraxis (Fig. 3). In the caudal hindbrain and spinal cord the depletion may be restricted to the FP itself (K.H., manuscript in preparation).

We detect the phenotype by the end of gastrulation (at 9–10 h after fertilization), several hours before the eye vesicles develop, as a flattening in the midline of the head anlagen. As the phenotype is zygotic this implies that the wild-type gene must normally function during gastrulation, when, in all vertebrates, inductive signalling that is essential for FP development may come from mesoderm, in particular the notochord^{12–15}. Mesoderm appears to gastrulate as usual in *cyc-1*, and its derivatives such as the notochord appear completely normal in size and form. Further, the paired hatching glands, derived from the prechordal plate¹⁶ that at early postgastrula stages underlies the brain, are normal in morphology and cell number.

To examine whether *cyc-1* affects FP induction we analysed genetic mosaics, made by injecting labelled wild-type cells into *cyc-1* mutants at the late blastula or early gastrula stage. We observed that wild-type ectoderm can differentiate into FP in *cyc-1* hosts, as judged by morphology (Fig. 4a, b) and labelling with zn-5 (not shown). On the other hand, wild-type mesoderm developing as notochord cannot induce the development of a FP in *cyc-1* hosts (Fig. 4c, d). These findings suggest that the mutation acts autonomously in ectoderm; the notochord appears unaffected.

The same analyses, however, revealed non-autonomous actions of *cyc-1* in the developing CNS. When wild-type cells transplanted into mutants subsequently differentiated as FP, adjacent mutant cells did so also (Fig. 4b). Thus mutant cells can develop as FP, showing that they possess the genetic machinery to do so, once they are so specified. A simple explanation of our findings is that two pathways for FP specification are present using distinct signalling factors and receptor systems. One of them, induction from the notochord, is blocked in *cyc-1*; the ectodermal cells might lack, for example, a receptor for the signalling factor. The second pathway, 'homeogenetic' induction¹⁷ between FP precursors, is intact. The latter pathway, recently observed independently in experiments with chick embryos⁵, might normally be important for organizing the FP as a coherent and discrete cellular community.

In a case in which wild-type FP cells were present in the midbrain of a mutant host, we observed local rescue of other cellular deficiencies in the ventral midbrain that characterize the mutant (not shown). Similarly, replacing mutant midventral diencephalic cells, where a FP is normally lacking, seems totally

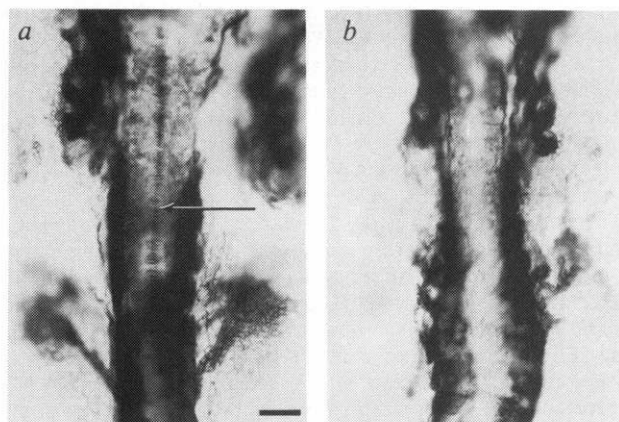
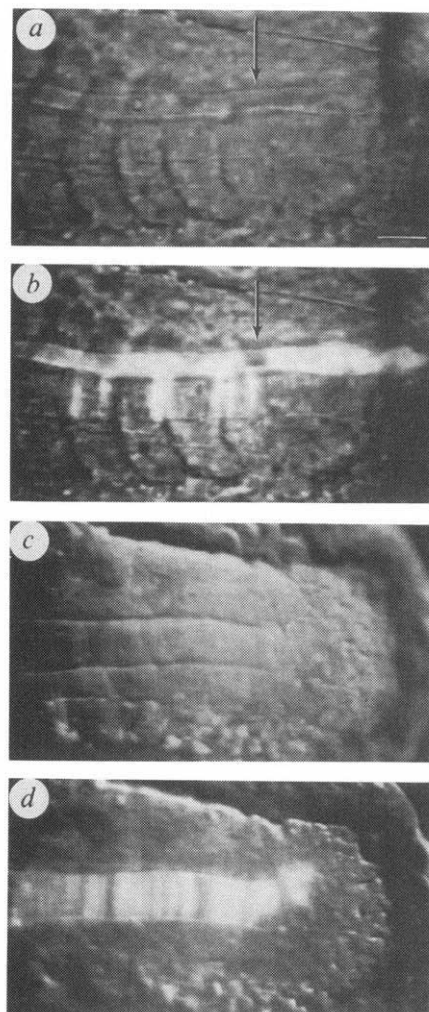


FIG. 3 *cyc-1* deletes the FP. Dorsal views of whole-mounted embryos immunochemically stained with the zn-5 monoclonal antibody⁷ at 28 h. The FP is labelled in the wild-type hindbrain and spinal cord (a, arrow) but is missing in *cyc-1* (b). Scale bar, 50 μ m.

FIG. 4 Mosaic analysis of *cyc-1* demonstrates autonomous and non-autonomous aspects of the mutant phenotype achieved by cell transplantation of wild-type FP precursors into mutant hosts at the early gastrula stage. Lateral left-side views of the tail of mutant hosts at 28 h. In panels *b* and *d* the implanted wild-type cells are made visible by fluorescent labelling. *a, b*, A mutant host in which the labelled wild-type cells differentiated as FP (arrows), and also locally induced the FP to develop from unlabelled mutant cells (arrow in *b*). In this example the mutant FP cells have wild-type FP neighbours, but in other cases the induction extended to short contiguous stretches of mutant cells. *c, d*, A mutant host in which the wild-type cells developed as notochord, but the FP was not induced by their presence. METHODS. Whole embryos were labelled as described in Fig. 1 and transplanted²⁴ at late blastula or the early gastrula stages to embryos at the same stage obtained from *cyc-1*/+ heterozygotes, either from natural crosses or by parthenogenesis by the EP method²⁵. Mutants were indistinguishable from their wild-type siblings at the time of transplantation, but were identified among the host embryos at 24 h. We did not study embryos in which we supposed, by the presence of wild-type cells in the fore- or midbrain, cyclopia may have been rescued. We observed no phenotypic rescue if the wild-type cells formed cell types other than the FP (*N*=15); in particular, wild-type notochord alone failed to rescue FP development (*N*=2). However, when wild-type cells were positioned above the notochord they differentiated into FP (*N*=6, as judged by morphology as in (*a*) and confirmed in three cases by zn-5 staining), and induced neighbouring mutant cells to differentiate as FP (confirmed in three cases by zn-5 staining). Scale bar, 20 μ m.



to rescue the forebrain defects, including cyclopia (K. H., unpublished). These findings suggest that ventral midline cells have an expanded organizational function in the brain as compared with the spinal cord, and might explain why the *cyc-1* phenotype is more severe rostrally. We note that cyclopia is a poorly understood congenital disorder in humans¹⁸, and could involve ventral midline CNS disturbances as in *cyc-1*, which serves as a reliable animal model for the disorder.

From these analyses we suggest that the primary deficiency in *cyc-1* is the single failure of specification of cells that come

to occupy the ventral midline of the neural tube along its entire length. The absence of this lineage, normally arising from median dorsal ectoderm of the gastrula, is reminiscent of dorsal group mutations¹⁹ in *Drosophila*, and of *single-minded*, a mutation that specifically deletes midline neural cells²⁰. The exceptionally simple organization of the FP in the zebrafish and the very discrete nature of the *cyc-1* defect will facilitate the identification of the molecular basis of the mutation, and the developmental roles that the midline cells have in organizing neighbouring cells and axon pathways^{21,22} in the developing CNS. □

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Dark-rearing delays the loss of NMDA-receptor function in kitten visual cortex

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SOME features of the visual cortex develop postnatally in mammals^{1,2}. For example, geniculocortical axons that initially overlap throughout cortical layer IV segregate postnatally into two sets of interleaved eye-specific bands^{3,4}. NMDA (N-methyl-D-aspartate) receptors are necessary for eye-specific axon-segregation in the frog tectum⁵, and as NMDA receptors play a greater part in synaptic transmission in early life^{6,7} and decrease in function during the period of axon segregation, they may be involved in the segregation of geniculocortical axons: they are well placed to do so as they transduce⁸ retinally derived signals essential for

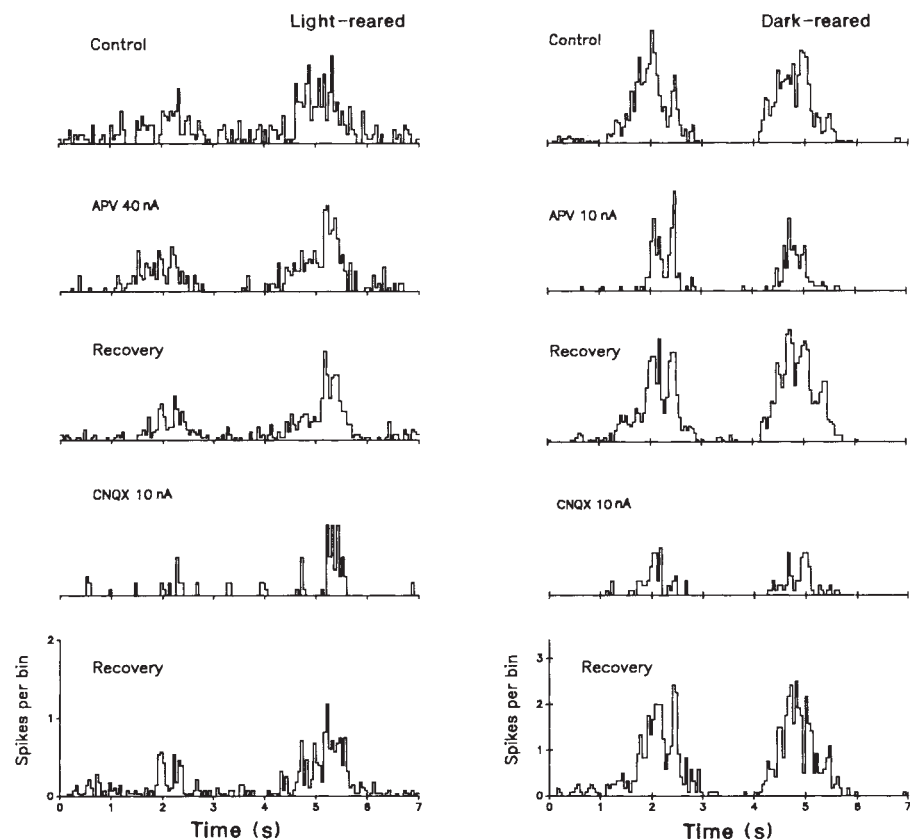
segregation⁹. Rearing animals in the dark in early life delays segregation^{10,11} and prolongs the critical period for plasticity¹². We now report that dark-rearing of kittens also delays the loss of NMDA receptor function in the visual cortex, supporting the view that they play an important part in neuronal development and plasticity.

We test the functional effectiveness of NMDA receptors with a physiological assay⁷. The NMDA component of the total visual response is estimated by blocking the receptors with the specific antagonist D-2-amino-5-phosphonopentanoate (D-APV) and measuring the resulting decrement in response firing rate during stimulation with a moving bar of light (Fig. 1).

To study NMDA receptors at the end of geniculocortical afferent segregation, we recorded from seven normal light-reared animals at 42–53 days old. The contribution of NMDA receptors to the visual response was greatest in layers II and III and minor or absent in layers IV, V and VI. On average, D-APV reduced visual responses to $48 \pm 28\%$ of control values in layers II and III ($n = 20$), with only two cells showing no change, whereas in deeper layers the visual responses of 24/31 cells were not significantly affected by D-APV (mean of $91\% \pm 11\%$ for layer IV and $85 \pm 17\%$ for layers V and VI). Figure 2 compares the functional distribution of NMDA receptors in 6-week-old light-reared kittens and adults. The distributions for layer IV and layer V/VI are not statistically different ($P > 0.2$), indicating that NMDA receptors have reached a mature functional distribution by the end of geniculocortical afferent segregation at 6 weeks.

FIG. 1. Right column, peri-stimulus time histograms for a layer IV cell recorded from an animal dark-reared to the day of recording at 47 days. The standard procedure was to measure the average response over 20 stimulus presentations and compare it with the response averaged over 8 stimulus presentations during the second and third minute of D-APV application using 10 nA ejecting current (second trace). If the averages were significantly different at the 0.05 level (t -test) and if the response recovered to within 75% of control levels within 15 min of ceasing drug application then the D-APV was judged to have had a significant effect. Most cells recovered from D-APV within 5 to 8 min as did this cell (middle trace). All drug applications were repeated at least once. Cells were discarded from the analysis if results from sequential tests differed greatly ($>20\%$), or if the control responses showed a coefficient of variation (c.v.) of more than 0.2 (for light-reared animals) or 0.25 for dark-reared animals. The c.v. stringency was relaxed for dark-reared animals because cells generally responded more variably. Application of CNQX using 10 nA ejecting current also reduced the visual response of this cell. Bin width, 50 ms. Left column, a layer IV cell recorded from a light-reared 44-day-old animal. In this case D-APV application fails to reduce the visual response of this cell, though applied with 40 nA ($4 \times$ standard trial) for 10 min. In general visual responses were not found to be further reduced by increasing the ejecting current to 20, 30 and 40 nA in cases where the standard 10 nA was ineffective. But spontaneous activity was affected by D-APV, and CNQX applied using 10 nA caused a reduction in the visual response. Recovery occurs within 10 min (bottom trace).

METHODS. Animals were anaesthetized with a mixture of halothane (0.7–1%) and N_2O/O_2 (2:1) and paralysed with pancuronium bromide (1.5 mg h^{-1}). Body temperature was maintained with a heating pad and CO_2 was kept



between 3.0–4.2%. Three barrel carbon fibre microelectrodes were used to record extracellular action potentials and iontophorese APV, CNQX and NMDA. Drugs were dissolved in distilled water at 20 mM and adjusted to pH 7.4 for D-APV, 8.5 for CNQX (Tocris, UK). Visual stimuli were presented on a tangent screen and moved continuously throughout control periods and drug trials under computer control (detailed methods have been published elsewhere⁷). Visual responses were expressed as average firing rate during the response minus spontaneous activity. A lesion was made to mark the layer location of each cell on completion of recording ($2 \mu\text{A d.c. for } 10 \text{ s}$, tip negative). Lesions were identified from sections stained with cresyl violet.

To study NMDA receptors under conditions of delayed segregation, six animals were reared in the dark from before eye-opening until the day of recording at 43–49 days of age. NMDA-dependent visual responses were found in all layers. D-APV applied with the same ejection parameters as used in the light-reared group significantly reduced the visual responses of 15/18 layer IV cells and 13/14 layer V/VI cells (mean of $40 \pm 25\%$ for layer IV and $58 \pm 16\%$ for layers V and VI). The distributions for deeper layers in 6-week-old dark-reared animals were significantly different from both 6-week-old and adult light-reared animals. The distribution for dark-reared animals was similar to that for 3-week-old normal animals, indicating that the distribution of NMDA receptors remains immature in dark-reared animals (Fig. 2).

Comparisons between the distributions shown in Fig. 2 support the idea that the normal decrease with age of visually activated NMDA receptors in layers IV, V and VI is arrested by dark-rearing. The NMDA component of the visual response was greater for layers IV, V and VI in the dark-reared kittens than in the light-reared animals of the same age. The distributions were significantly different when layer IV ($P < 0.001$) and layers V/VI ($P < 0.001$) were compared for light- and dark-reared groups.

Control experiments suggest that D-APV was being ejected at sufficient concentrations in cases where it did not affect visual responses. Using an ejecting current fourfold that needed to reduce the visual response of layer IV cells in the dark-reared group was ineffective on layer IV of the light-reared group (Fig. 1). Moreover, excitation induced by iontophoresis of NMDA could be antagonized by D-APV even when D-APV had no effect on the visual response. Also, the standard drug ejection protocol used for each cell resulted in drug delivery that was similar for both groups when just layer II/III cells were compared ($P > 0.2$).

We also tested whether rearing in the dark decreased the non-NMDA receptor component of the response. Application

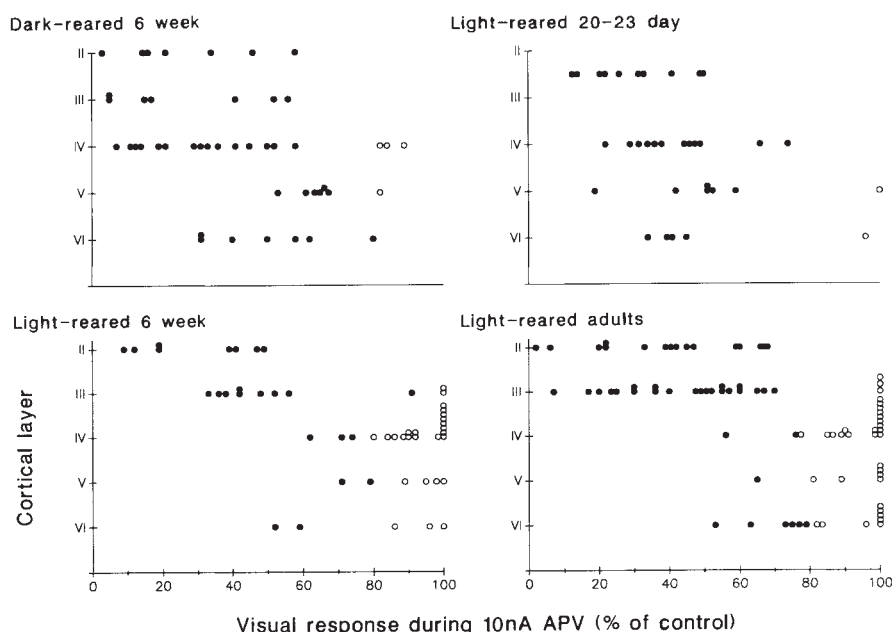
of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA antagonist, using 10 nA ejecting current, reduced the visual responses of every cell studied in both groups (33/33). There was no statistical difference between the effectiveness of CNQX in light- and dark-reared animals ($P > 0.5$) and suppression of visual responses was independent of layer in both groups (average reduction to 30% of control). Hence, cells in layer IV of light-reared animals which were unaffected by D-APV (even when the dose was increased to 40 nA for 10 min) were antagonized by CNQX ejected with 10 nA for 2–3 min (see Fig. 1). This also confirms that visually driven synapses were within diffusion range of the electrode.

The greater effectiveness of D-APV in dark- compared with light-reared animals is unlikely to be due to differences in the vigour of response between the two groups. As reported by others, cells are less responsive in dark-reared animals than in light-reared^{11,12} (mean of 10 ± 6 spikes per second compared with 19 ± 11 , averaged over the response duration). But (in agreement with previous studies^{7,13}) we found no correspondence between the effectiveness of D-APV and firing rate for individual cells. Similarly, previous studies have shown that the percentage reduction in visual response caused by D-APV is the same for great and small responses evoked in the same cells by high and low contrast stimuli¹³.

Our results demonstrate a close correlation between the capacity for geniculocortical afferents to segregate and the presence of visually driven NMDA receptors in layer IV under conditions of either normal development or dark-rearing. These results support the hypothesis that NMDA receptors are necessary for eye-specific afferent segregation in kitten visual cortex, as they are in frog tectum^{5,14}. NMDA receptors may be activated preferentially by correlated inputs as they are voltage-dependent in visual cortex¹⁵; they could thereby provide a mechanism for sorting highly correlated synapses driven by the same eye versus lesser correlated inputs driven by different eyes¹⁶. During normal

FIG. 2 Average firing rate during application of D-APV is plotted for each cell as a percentage of the control firing rate according to the layer in which the cell was recorded (see Fig. 1 for methods). Each point represents data from a single cell. Points occurring on the extreme right (100%) represent cells that showed no change in reply to D-APV. Filled circles represent responses that were significantly changed from control ($P \leq 0.05$, *t*-test; degrees of freedom, 6 or 7), open circles show cases where effects were insignificant ($P \geq 0.1$). Top left, 46 cells recorded from 6 animals dark-reared until the time of recording at 43–49 days. The distribution for layer IV is significantly different from that of the 6-week light-reared animals ($P < 0.001$, two-tailed *t*-test); distributions differ even if the data are pooled for all layers ($P < 0.001$). Lower left, 50 cells recorded from 7 animals light-reared to 42–53 days of age. Lower right, 57 cells recorded from 15 animals between 7 months and 3 years of age. The distributions for layers IV, V and VI combined are not significantly different between 6-week light-reared and adult animals ($P > 0.2$); the percentage of cells that were not significantly affected is almost identical (77 compared with 80%). Top right, results from 34 neurons recorded from five 3-week-old animals are included for comparison. Overall, layer IV shows a similar sensitivity to treatment with D-APV in dark-reared 6-week animals to that shown by 3-week light-reared animals (average of $40\% \pm 26$ and $43\% \pm 14.5$, respectively). Results for layers II and III are combined because they are difficult to distinguish at this age¹.

METHODS. Four litters were reared in light-tight cages in a darkened room starting at 4–5 days post-partum until 43–49 days. Animals were sedated with Acepromazine (AVCO, Fort Dodge, Iowa) before being removed from



the dark on the day of the recording. Animals were transported in a dark box before anaesthesia was induced. The period of unanaesthetized light exposure was less than 5 or 10 min on the day of the experiment. Because it takes ~40 h of light exposure to produce segregation¹⁰, this short exposure is unlikely to have affected our results. The experiments usually lasted about 16 h, but as anaesthesia prevents plasticity in visual cortex²², little change was expected or observed.

development the progressive downregulation of this mechanism would bring segregation to a close and prevent further plasticity in this layer. The arrest of NMDA-receptor downregulation by dark-rearing would therefore explain the capacity for segregation on subsequent exposure to the light and might provide a basis for the extension of the critical period.

Our results demonstrate that sensory experience rather than a 'genetic clock' causes the decrease in NMDA-receptor function seen with normal rearing. But dark-rearing does not seem to preserve NMDA-receptor function by affecting numbers either of channels^{17,18}, which decrease with dark-rearing, or of receptors¹⁹, which are unaffected. NMDA receptors may migrate from under the visually driven synapses during light-rearing or alternatively one of the many regulation sites on the NMDA receptor could alter, for example changing its phosphorylation state²⁰ or glycine affinity²¹, and hence its function. □

was low. We have also used quantal analysis to show that synaptic depression following prolonged stimulation at these synapses is primarily a presynaptic phenomenon.

Conventional intracellular microelectrode voltage recordings were made in the CA1 subfield of slices of rat hippocampus for 60 neurons. Precautions were taken to reduce spontaneous synaptic and epileptiform activity (see legend to Fig. 1). Large numbers of trials of small (<1 mV) excitatory post-synaptic potentials (e.p.s.ps) were evoked at 0.5–5 Hz and their peak amplitudes were displayed as amplitude histograms. The data were scanned for periods of stability, from which epochs of 400–1,800 consecutive trials were selected. Epochs yielding

TABLE 1 Summary of the MLE results for 25 e.p.s.ps for which the visual measurement and MLE procedures were in close agreement

E.p.s.p.	Number of trials	Mean quantal amplitude ±s.d. (μV)	Optimum s.d. (μV)	Measured baseline noise s.d. (μV)
AH3	500	137 ± 13	45	76
AH4	500	107 ± 9	35	79
AH6	1,200	160 ± 34	70	183
AH7	500	88 ± 12	30	57
AH8	800	152 ± 6	80	145
AH9	600	139 ± 24	60	153
AH11	600	111 ± 19	40	97
AH15	1,800	197 ± 35	100	155
AH23	1,000	160 ± 12	60	115
AH24	800	118 ± 14	35	90
AH28	500	154 ± 26	50	172
AH32	600	180 ± 19	75	92
AH39	500	137 ± 19	55	69
AH40	500	152 ± 4	65	67
AH41	600	122 ± 16	45	83
AH42	500	126 ± 6	45	81
AH43	600	151 ± 42	60	122
AH44	500	142 ± 35	55	73
AH46	1,100	100 ± 16	35	64
AH48	500	136 ± 6	55	98
AH63	400	84 ± 18	30	64
AH78	600	123 ± 35	60	102
AH83	800	104 ± 17	40	52
AH86	400	85 ± 19	35	47
AH88	600	113 ± 25	40	52

The number of trials refers to those used to construct the amplitude histogram on which the analysis was based, not the total number recorded for each e.p.s.p. The optimum standard deviation (s.d.) is the s.d. associated with each discrete amplitude of the optimal MLE solution. The measured baseline noise s.d. is that of the noise distribution measured during the pre-stimulus period of each trial. For most e.p.s.ps, similar MLE solutions could be obtained using s.d.s considerably higher than the optimal; however, in every case, the histograms were fitted best using an s.d. lower than that of the baseline noise. Several factors may contribute to this discrepancy. A proportion of the baseline noise may be caused by the enhanced spontaneous release of quanta from synapses undergoing repetitive stimulation^{27,32}, which would contribute less noise during the evoked e.p.s.p. than during the baseline period³¹. Our analysis and simulations of the baseline noise indicated that, under the pharmacological conditions used here, the rates of spontaneous transmitter release were low, such that enhanced spontaneous release from a very small number of synapses could generate the additional noise observed in the baseline. Evidence that such a mechanism operates here comes from experiments in which the baseline noise was found to increase during 5 Hz stimulation and slowly return to initial levels when stimulation was stopped. The discrepancy in variance levels was also found to be less when much slower stimulation rates were used. Additionally, although the measured noise distributions were roughly gaussian, most showed positive skewness and kurtosis, such that their peaks were sharper than their s.d. values would indicate. Simulations suggest that the width of the noise distribution near its peak (rather than its base) is important in determining the shape of the e.p.s.p. amplitude histogram peaks and hence the resolution of the MLE procedure¹⁴.

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Quantal analysis of excitatory synaptic action and depression in hippocampal slices

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QUANTAL analysis can provide a quantitative description of important aspects of chemical synaptic transmission and its modification^{1–3}. The technique has recently been applied to excitatory synapses within the hippocampus^{4–10}, especially the form of synaptic plasticity known as long-term potentiation^{11–13}. However, these attempts have met with only limited success, in that the individual quantal amplitudes making up the synaptic response generally could not be resolved. Here we have paid attention to the possible instability of the quantal fluctuation pattern over time. We were able to resolve individual quantal component amplitudes for a high proportion of the experiments, and so demonstrate the quantal nature of excitatory transmission in the CA1 region of the hippocampus. Mean quantal amplitudes for individual excitatory postsynaptic potentials were 84–197 μV, with a mean of 131 ± 29 μV. For periods during which the fluctuation pattern was stable, the variance associated with individual quantal amplitudes

histograms with clear and regularly spaced peaks were obtained for 38 of the 60 cells examined (Fig. 1a). Such epochs were usually interspersed with periods for which the e.p.s.p. fluctuation pattern varied, possibly as a result of short-term changes in quantal amplitude. The higher stimulus rates seemed to be more successful in producing stable epochs of data.

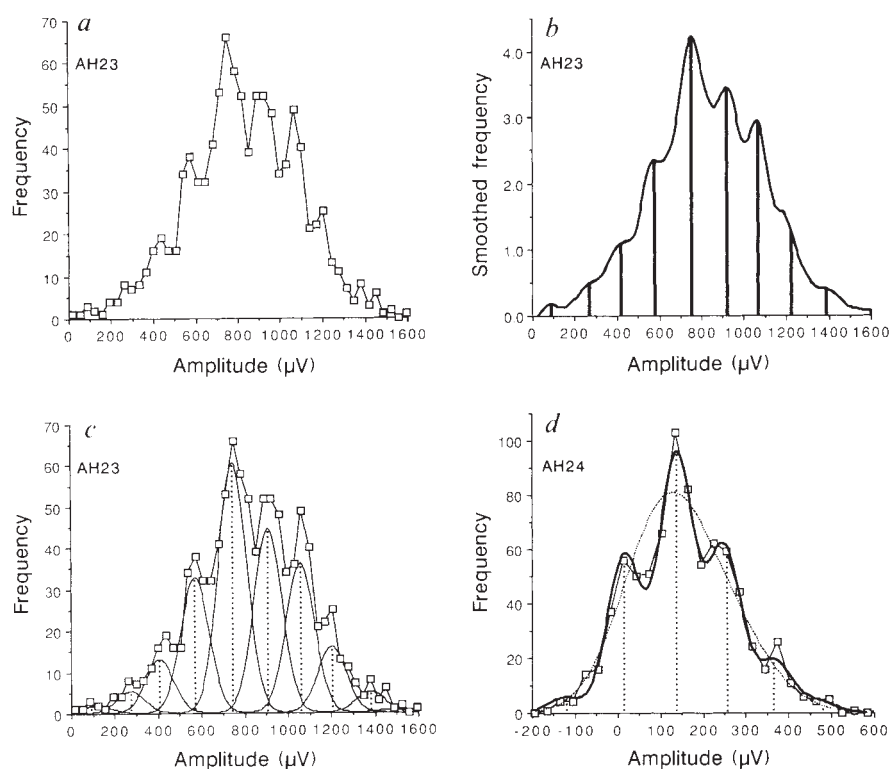
The existence of equally spaced peaks in the amplitude histograms is strong evidence for the quantal nature of transmission at these synapses. The mean quantal amplitude, representing the postsynaptic effect of the release of a single quantum of transmitter, was estimated in two ways. First, the selected histograms were binned finely and smoothed (Fig. 1b). The locations of the main peaks were measured visually, and their mean separation, corresponding to the mean quantal amplitude, was calculated. Second, each histogram was analysed using a maximum likelihood estimation algorithm¹⁴ (MLE) to determine the optimal pattern of component amplitudes, their prob-

abilities and associated variance (Figs 1c, d and 2a). For 25 of the 38 e.p.s.ps the visual and MLE solutions were in close agreement. The mean quantal amplitudes ranged between 84 and 197 μV , with a mean of $131 \pm 29 \mu\text{V}$ (Table 1).

Taking this mean quantal amplitude together with the average e.p.s.p. time course, and a conventional value for CA1 neuronal input resistance of 25 M Ω (ref. 15), it can be calculated that the charge reaching the soma for a single quantum is of the order of 80 fC. This is in line with the modal value for the fast component of miniature synaptic currents observed by Bekkers and Stevens⁹ of 50–100 fC. These authors calculated that this value represented the opening of 30–110 channels. Synaptic vesicles are each likely to contain $\sim 4,000$ glutamate molecules^{16,17}. Although two glutamate molecules may be required to open each channel^{18,19}, it seems likely that a single synaptic vesicle contains ample transmitter to saturate the postsynaptic receptors involved in generating a quantal e.p.s.p. The quantal

FIG. 1 a, Amplitude histogram showing the distribution of e.p.s.p. peak amplitudes during a selected epoch of 1,000 consecutive trials. The histogram shows a succession of clear peaks, indicative of an underlying quantal release process. The peaks corresponding to larger e.p.s.p. amplitudes seem to be no less sharp than those for smaller amplitudes, suggesting that the quantal variance is low. The precise shapes and locations of the peaks are, however, dependent on the bin width used (in this case 35 μV). b, Amplitude histogram based on the same data as in a, but using a narrow bin width (5 μV) and smoothed using a moving gaussian digital filter. Changing the characteristics of the filter affects the sharpness of the peaks but has little effect on their locations. Vertical bars show the locations of the apices of the peaks as determined visually. The spacing of the bars, representing the mean quantal amplitude for this e.p.s.p., was $158 \pm 15 \mu\text{V}$. c, The same amplitude histogram as in a but showing the combination of discrete amplitudes (vertical dotted lines) and gaussian distributions as determined by the MLE procedure. The s.d. of the gaussian distributions giving the best fit to the data was 60 μV , compared with the s.d. of the baseline noise distribution of 115 μV . The mean separation between discrete amplitudes was $160 \pm 12 \mu\text{V}$, in good agreement with the visual measurement procedure shown in b. d, Amplitude histogram for 800 successive trials of another e.p.s.p., unfiltered and using a bin width of 30 μV . The vertical dotted lines show the locations of the discrete amplitudes as determined by both visual and MLE analysis procedures, which were in good agreement. The mean quantal amplitude in this case was $118 \pm 14 \mu\text{V}$. The reconvolution of these discrete amplitudes with gaussian distributions of s.d. 35 μV , which gave the optimum fit to the data, is shown by the thick solid line. The dotted curve shows the reconvolution for the MLE solution using the s.d. of the baseline noise (90 μV), which gave a less satisfactory fit to the data, showing no obvious peaks associated with each discrete amplitude level.

METHODS. Transverse, 400 μm thick, hippocampal slices were prepared by conventional methods from young adult albino rats (Sprague-Dawley strain; 140–200 g) and maintained in an interface-type recording chamber at 34 °C. The artificial cerebrospinal fluid used contained 119 mM NaCl, 3.5 mM KCl, 1 mM NaH_2PO_4 , 4 mM MgSO_4 , 4 mM CaCl_2 , 26 mM NaHCO_3 , 11 mM glucose. Various steps were taken to reduce the noise caused by spontaneous synaptic activity, including the use of elevated Ca^{2+} and Mg^{2+} levels and the removal of the CA3 region³³. For most experiments the artificial cerebrospinal fluid included picrotoxin (100 μM), APV (50 μM), and glutamine (1 mM). Intracellular recordings were made from *stratum pyramidale* of CA1 using electrodes containing 2M potassium methylsulphate. E.p.s.ps were evoked by a bipolar wire electrode placed at varying positions on the slice. The intensity of stimulation was adjusted to obtain the smallest e.p.s.p. that appeared to be evoked reliably and was then left unchanged. Between 1,000 and 12,000 trials were evoked at 0.5–5 Hz (usually 3 or 5 Hz) and digitized



at 5 KHz. The e.p.s.p. peak amplitudes on each trial and the baseline noise were measured⁶ and displayed as frequency histograms. Most e.p.s.ps were obviously non-stationary, with periods of data yielding stable amplitude histograms being interspersed with periods for which the histograms were smooth or showed irregular patterns of peaks. Epochs of 400–1,800 consecutive trials were therefore selected on the basis of yielding histograms with clear, regularly-spaced peaks whose locations remained constant when the epoch was subdivided. These histograms were each analysed in two ways and the results compared. First, the histograms were binned finely, smoothed using a moving gaussian filter and the locations of the peaks measured visually. Second, a maximum likelihood estimation algorithm (MLE) was used to determine the pattern of component amplitudes, probabilities and associated variance that provided the best fit to each finely-binned, unsmoothed histogram. Our MLE procedure required no assumptions about the statistics underlying release probabilities or about the location or spacing of amplitudes, other than that the fluctuation pattern is composed of a limited number of discrete amplitudes. Single gaussian noise models were used in the MLE procedure. As the histogram peaks generally appeared sharper than would be predicted from the measured baseline noise, the MLE analysis was repeated for a range of noise variance levels and the solution giving the best statistical fit to the histogram was selected.

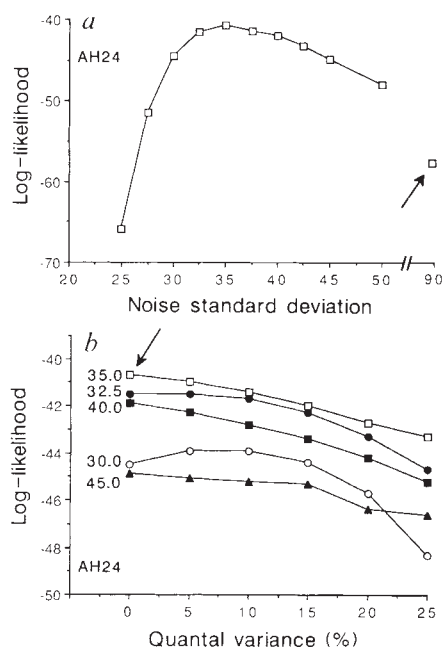
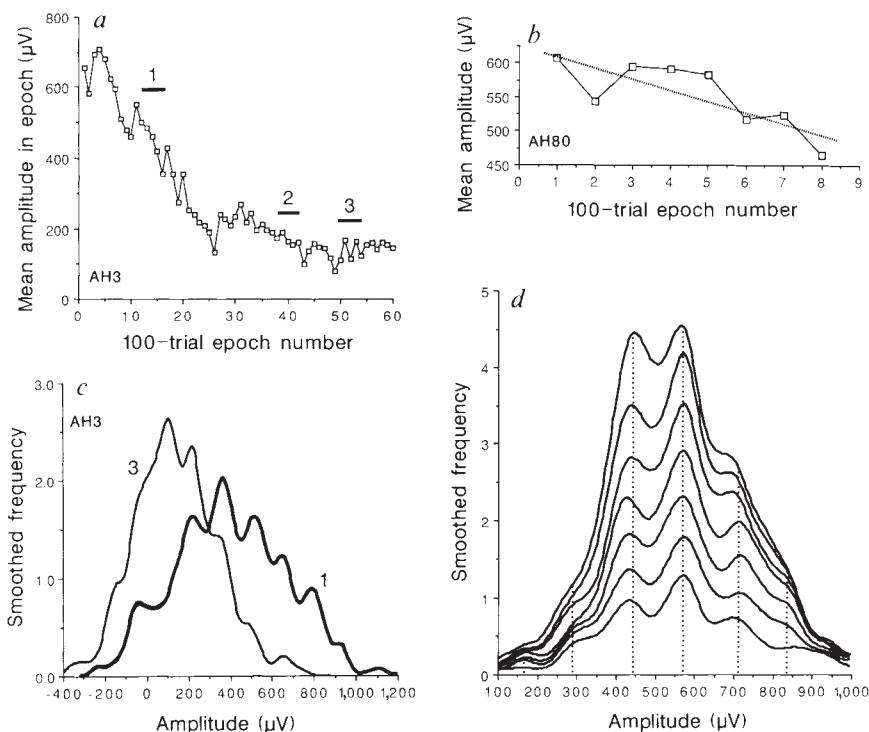


FIG. 2 Graph showing the normalized log-likelihood value, an indication of the goodness of fit, for MLE solutions using gaussian distributions with a range of standard deviations, for the e.p.s.p. from Fig. 1d. The log-likelihood shows a single, clear maximum, indicating the existence of a unique optimal fit to the data³⁴, at an s.d. of 35 μV . The log-likelihood value obtained using the s.d. of the measured baseline noise (90 μV) is shown arrowed, and clearly represents a less good fit to the data. *b*, Graphs showing the log-likelihood values for the same e.p.s.p. for MLE solutions using a range of quantal variances for each of several overall variance values (s.d. in μV indicated to the left of each curve). The overall variance was divided into two components, one of which was constant for all discrete amplitudes, representing the contaminating noise, while the other component, representing the variance associated with each quantum, was scaled linearly with the number of quanta comprising each discrete amplitude. The quantal variance is expressed as a percentage of the overall variance. The optimal solution had an overall s.d. of 35 μV with zero quantal variance (arrowed). However, the curves, particularly those for s.d.s close to the optimal, are fairly flat (note different scale to *a*), indicating that the solutions are relatively insensitive to these levels of quantal variance. The existence of much higher levels of quantal variance appears unlikely in view of the approximately equal sharpness of peaks on both the left and right sides of most amplitude histograms.

amplitude would then be limited by the number of receptors available, and the variance associated with quanta at a single release site should be low, unless the number of functionally responsive receptors changes. The shapes of our amplitude histograms, during periods of relative stability, are certainly compatible with this suggestion. Peaks corresponding to the release of several quanta were as sharp as those corresponding to the release of only one or two. For a sample of 13 e.p.s.p.s, the MLE analysis was repeated incorporating various levels of

quantal variance, and in each case the best fits to the data were obtained using zero or very low levels of quantal variance (Fig. 2*b*). In this respect, our results are in contrast to the findings of Bekkers and Stevens^{9,10,20}. These workers reported very high levels of variance (c.v. 42–55%) of miniature synaptic currents for hippocampal cells in culture and in slices, and their histograms for evoked synaptic current amplitudes did not show clear peaks. It may be that quantal events evoked in younger animals or by hypertonic solutions show more variation than

FIG. 3 *a*, Graph showing the decrease in e.p.s.p. mean peak amplitude, shown as 100-trial averages, during prolonged stimulation at 5 Hz. The decrease shows an approximately exponential time course from an initial value of approximately 650 μV to 150 μV over 6,000 trials. Note that there is also considerable short-term variation between 100-trial averages, which may reflect short-term changes in the quantal amplitude. *b*, Graph showing the variation in 100-trial mean peak amplitude for another e.p.s.p. over a 800-trial epoch, stimulated at 1 Hz. The mean shows both short term variation and a downward trend. Broken line is the least-squares regression ($r^2=0.629$) which shows a decline in peak amplitude of 16 μV per 100 trials over this period. *c*, Two smoothed amplitude histograms, each of 500 trials, taken from epochs 1 (thick line) and 3 (thin line) indicated by bars in *a*. The mean amplitude of the e.p.s.p. was 402 μV during epoch 1, 170 μV during epoch 2 (histogram not shown) and 144 μV during epoch 3. The results of both the visual measurement and MLE analysis procedures gave values for mean quantal amplitude of 137 ± 10 μV for epoch 1, 143 ± 15 μV for epoch 2 and 136 ± 19 μV for epoch 3. Thus the mean quantal amplitude did not change significantly while the e.p.s.p. mean amplitude decreased by a factor of more than two, suggesting a presynaptic locus for the depression during this period. *d*, Smoothed cumulative amplitude histograms (100-trial increments from 200) for the epoch shown in *b*, showing clear peaks in spite of the downward trend in e.p.s.p. amplitude. The cumulative histograms show that the locations of the peaks remain constant throughout, but their relative heights change. Peaks corresponding



to smaller e.p.s.p. amplitudes become relatively higher at the expense of those corresponding to larger e.p.s.p. amplitudes.

those arising from physiological neuronal activity in preparations from adult animals. Also the considerable instability of quantal amplitudes over time that we observed might contribute to apparent high levels of quantal variance and obscure peaks in histograms. Evidence for low quantal variance, small numbers of postsynaptic receptors and the likely saturation of receptors by a single quantum of transmitter has been obtained for inhibitory synapses in the dentate gyrus²¹.

A complete quantal analysis also involves determining, if possible, the statistical distribution describing the number of quanta released from trial to trial. Recent evidence from both the neuromuscular junction²² and the spinal cord^{23–25} indicates that different sites release quanta with different probabilities, requiring the use of compound rather than simple binomial statistics. We have analysed the MLE solutions for the 15 e.p.s.ps whose mean showed no trend during the epoch analysed. A simplex optimization routine was used to determine the optimal Poisson, simple binomial and compound binomial distribution that matched the probability density of the MLE solution for each e.p.s.p., and the different optima were compared using χ^2 statistics. Two e.p.s.ps showed a bimodal distribution and six further e.p.s.ps did not correspond closely to any of these distributions. Of the remainder, two were approximately fitted by Poisson distributions, one by simple binomial and four by compound binomial distributions. We conclude that it is unsafe to assume that Poisson or simple binomial statistics will be generally appropriate for these synapses.

During prolonged repetitive stimulation, most e.p.s.ps showed a roughly exponential decrease in mean peak amplitude (Fig. 3a), a phenomenon known as synaptic depression^{26–28}. Interestingly, we were unable to induce long-term potentiation (LTP) (in the absence of 2-amino-5-phosphonovaleric acid) after prolonged stimulation at the higher rates¹³. We investigated whether the decrease was due to a reduction in the quantal amplitude or in the number of quanta released on each trial. On 10 occasions it was possible to obtain histograms showing clear peaks from epochs of data during which the mean peak amplitude of the e.p.s.p. decreased (Fig. 3b). When these histograms were displayed cumulatively, the locations of the peaks were constant throughout, but their relative heights changed (Fig. 3d). For three e.p.s.ps, it was possible to obtain two or more separate epochs of 500 or more trials for which the mean separation between peaks remained constant while their relative heights changed and the mean amplitude decreased (Fig. 3c). These results point to this form of depression being primarily the result of a reduction in the number of quanta released per trial and, as such, a presynaptic phenomenon^{26–29}. However, we were generally unable to obtain clear histograms with stationary peaks from epochs including the first 100 or 200 trials after the start of stimulation. Our preliminary analysis of these early periods suggests that the quantal amplitude often decreases during this phase. This, together with the instability of the fluctuation pattern, suggests that the quantal amplitude is potentially variable over time. Given the likelihood of receptor saturation, this variation may arise from changes in the numbers of functionally responsive receptors available, for example as a result of varying levels of receptor desensitization induced by activity-dependent changes in extracellular glutamate concentration^{18,19,30,31}.

Our results have at least three clear implications for the study of plasticity at hippocampal synapses. First, the likelihood of postsynaptic receptor saturation means that some forms of enhanced transmitter release, such as those involving an increase in the number of transmitter molecules released at a single site, would not produce an increase in the efficacy of the synapse. Second, short-term changes in quantal amplitude may occur unpredictably. Third, the statistical distribution is unlikely to be a simple binomial or Poisson for all junctions. This indicates that although simple indices of synaptic variability such as mean²/variance are capable of demonstrating, for example, that

an increase of release probabilities contributes to LTP^{8,10}, a more complete quantal analysis will be required to quantify the relative importance of pre- and postsynaptic changes. □

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Two types of orientation-sensitive responses of amacrine cells in the mammalian retina

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NEURONS sensitive to the orientation of light stimuli exist throughout the mammalian visual system^{1–3}, suggesting that this spatial feature is a fundamental cue used by the brain to decipher visual information. The most peripheral neurons known to show orientation sensitivity are the retinal ganglion cells. Considerable morphological^{4,5} and pharmacological^{6–10} data suggest that the orientation sensitivity of ganglion cells is formed, at least partly, by the amacrine cells, which are laterally oriented interneurons presynaptic to the ganglion cells in the inner plexiform layer. So far there have been few studies of the responses of amacrine cells to oriented visual stimuli and their role in forming orientation-sensitive responses in the retina remains unclear. Here I report the novel finding of a population of amacrine cells in the rabbit retina which are orientation-sensitive. These amacrine cells can be divided into two subtypes, whose orientation sensitivity is manufactured by two distinct mechanisms. The orientation sensitivity of the first subtype of amacrine cell is formed from the interactions of excitatory, centre-receptive field synaptic inputs and inhibitory inputs of opposite polarity, whereas that for cells of the second subtype seems to be the product of a marked asymmetry in their dendritic arbors.

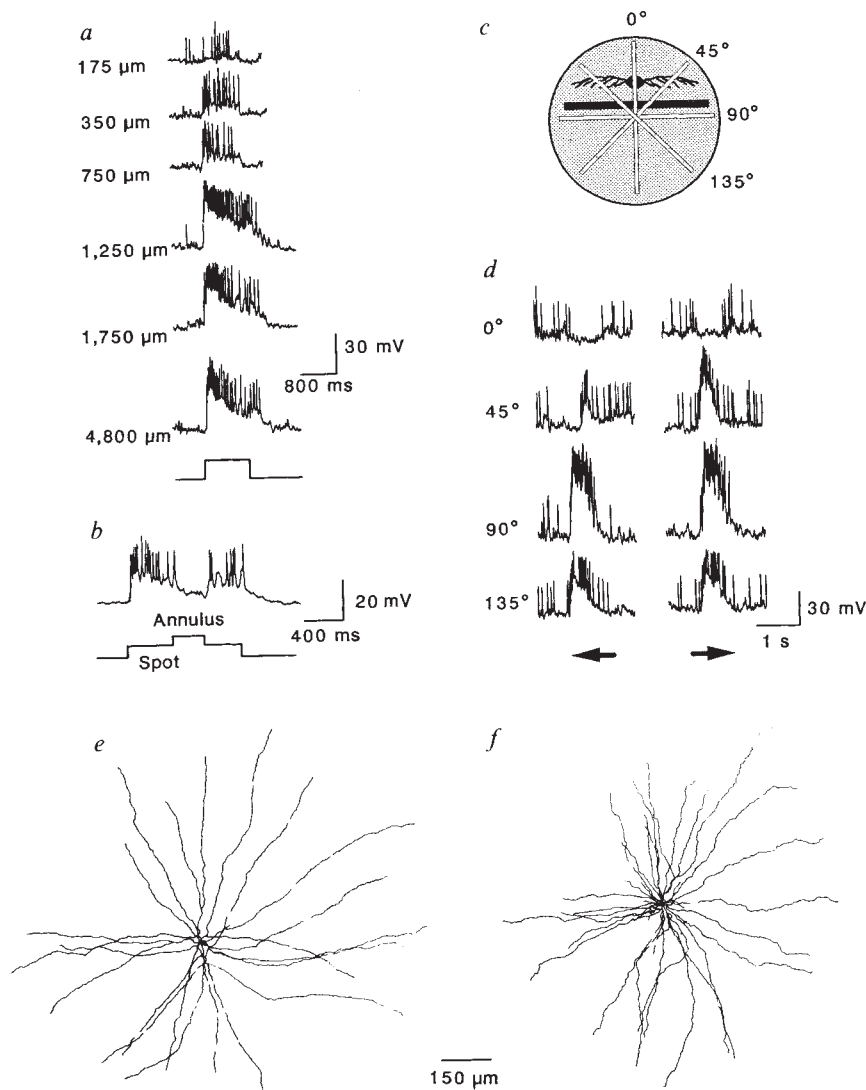
The receptive fields of amacrine cells were measured with concentric spots of light of increasing size (Fig. 1a). The on-centre amacrine cell whose responses are shown in Fig. 1 displayed an extraordinarily large receptive field of around 1,250 μm , corresponding to the smallest spot size that produced a saturating response. An antagonistic surround response was evoked by presentation of an annulus of light against an adapting small spot (Fig. 1b). A 95 μm -wide slit of light oriented at 90° (parallel to the visual streak) produced a maximum amplitude on-centre response consisting of high frequency spiking atop a large depolarization (Fig. 1d). After rotation of the slit to 0°, an inhibitory response was produced as seen by the cessation of spike activity coupled with a hyperpolarization of the mem-

brane potential. The hyperpolarization showed a reversal potential negative to the dark membrane potential (DMP) indicative of an inhibitory rather than a disfacilitatory mechanism. Amacrine cells for which rotation of a slit of light 90° from the preferred orientation (that most effective in producing an excitatory, centre-receptive field response) produced clear inhibition of the opposite polarity have been termed orientation-selective. Mechanistically, the selectivity of these cells arises apparently from the interplay between excitatory and inhibitory events. Several models have been proposed to explain how the interactions of synaptic inputs can create orientation selectivity of visual neurons^{1,8,11,12}. Stationary slits of light were as effective as moving slits in evoking orientation-selective responses,

FIG. 1 Intracellularly recorded responses of an orientation-selective amacrine cell to light stimuli. *a*, Responses to concentric spots of light, aligned about the tip of the microelectrode before cell impalement, used to measure receptive field size. The value to the left of each response indicates the diameter of the spot on the retinal surface. The upward and downward deflection of the line at the bottom (light trace) indicates the onset and offset of a step of light. Light intensity is $\log -4.0$. *b*, Response to small spot and annulus of light showing antagonistic, centre-surround receptive field. A 350 μm small spot of light elicited an on-centre response from this cell, whereas the superimposed annulus (inner diameter 350 μm , outer diameter 6.2 mm) evoked a response of opposite polarity. *c*, Diagram of rabbit retina showing four angles of orientation used for rectangular slit of light (white bars). Optic disk (dark oval), medullary rays (branched lines) and visual streak (dark bar) are indicated. Slits of light on the retina are not drawn to scale. *d*, Response of cell to rectangular slit of light (95 μm wide and 6.2 mm in length) moving back and forth (direction indicated by arrows at bottom) across retina at a speed of 1.3 mm s^{-1} . This cell is excited when the stimulus is oriented at 90° and inhibited when the stimulus is rotated to 0°. *e*, Flatmount-view drawing of horseradish peroxidase (HRP)-labelled amacrine cell whose responses were shown above. Orientation of the visual streak in this and all subsequent drawings is horizontal across the page. *f*, Flatmount-view drawing of another HRP-labelled orientation-selective amacrine cell from rabbit.

METHODS. The general methods used during this study have been reported previously²³. Briefly, adult dutch-belted rabbits were anaesthetized with ethyl carbamate injected intraperitoneally. The orbit and surrounding tissue were anaesthetized locally with lidocaine hydrochloride, after which the eye was removed. The eye was hemisected, the anterior portions discarded, and the resultant retina-eyecup mounted in a superfusion chamber. The retina-eyecup was superfused with a modified Ame's medium (Sigma) to which 25 mM NaHCO_3 and 10 mM glucose were added. The superfusate was maintained at 35 °C, saturated with a gaseous mixture of 95% O_2 - CO_2 to a final pH 7.4, and delivered to the chamber at a rate of 30 ml min^{-1} .

Light stimuli were presented to the surface of the retina via a dual beam optical bench; maximum light intensity for both beams ($\log 0.0$) = 3.41×10^{14} quanta $\text{cm}^{-2} \text{s}^{-1}$. Stimuli consisted of spots, annuli, or rectangular slits of light. The slit was either stationary or moving at speeds of 0.8–20 mm s^{-1} across the retinal surface with a total excursion of 6.2 mm. Intracellular recordings were obtained with glass micropipettes filled with 8% HRP (Boehringer-Mannheim, grade I) dissolved in 0.5 M potassium acetate and 0.1 M Tris buffer (pH 7.6) and then backfilled with 4 M potassium chloride to a final d.c. resistance of 250–600 M Ω . Recordings were amplified with a high-impedance amplifier (Axon Instruments), digitized and stored on



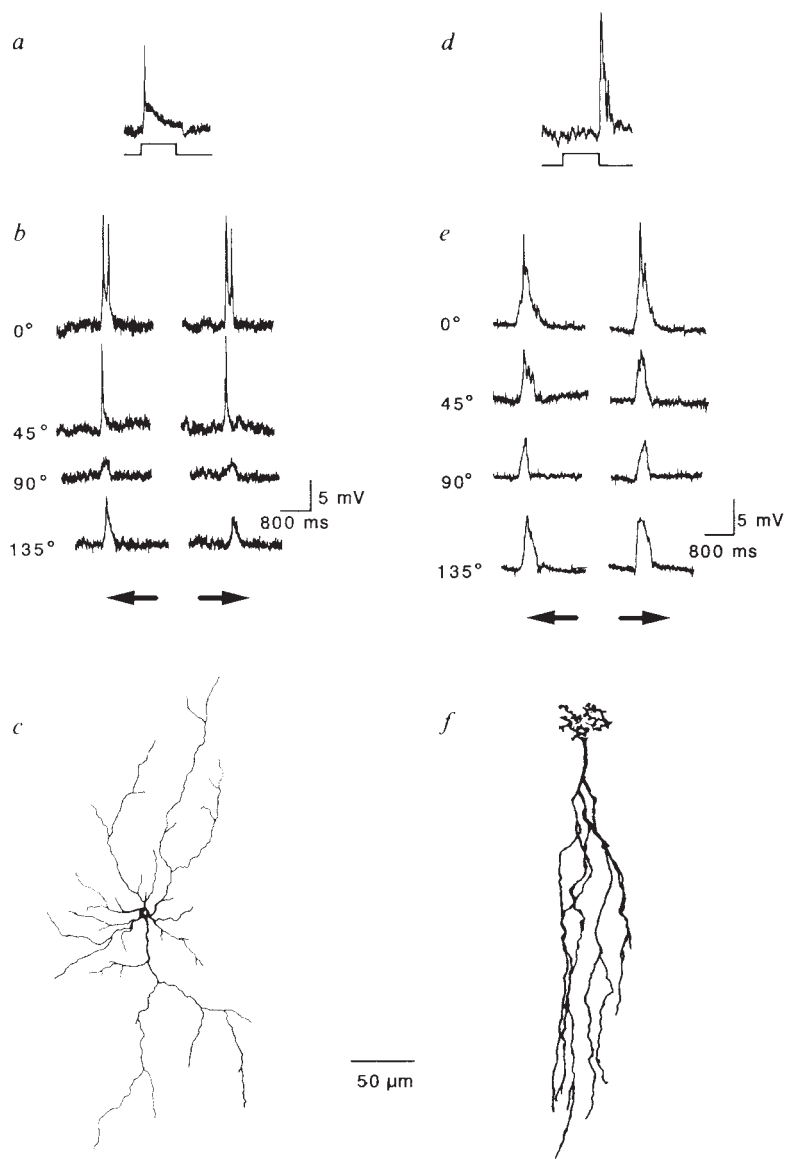
computer. After physiological characterization, cells were iontophoresed with HRP using a combination of d.c. and sinusoidal currents of 0.2–0.7 nA. Retinas were processed histologically using 3,3'-diaminobenzidine tetrahydrochloride or benzidine dihydrochloride as chromagens. Cells were then photographed under the microscope and drawn with the aid of a camera lucida. All cells in this study were labelled with HRP and identified morphologically. Identification of neurons as amacrine cells was based on position of the soma in the retina, clear lack of an axon, and similarities in dendritic architecture to amacrine cells described in morphological and/or immunocytochemical studies.

indicating that the angle of the slit and not its motion was the primary stimulus. Orientation-selective amacrine cells showed DMPs of -47.8 ± 6.6 mV (mean $\pm$ s.d. for 5 cells), and totalled 11% of all amacrine cells studied (5 of 44 cells). They were either on- or off-centre, transient or sustained, showed large centre-receptive fields about $1,250 \mu\text{m}$ in diameter with antagonistic surrounds and preferred light stimuli oriented parallel or orthogonal to the visual streak.

Orientation-selective amacrine cells labelled with horseradish peroxidase had a number of morphological features in common (Fig. 1e and f). These neurons were all within the visual streak and had small cell bodies, $8\text{--}12 \mu\text{m}$ in diameter, in the proximal region of the inner nuclear layer. They displayed large, symmetrical dendritic fields, $900\text{--}1,200 \mu\text{m}$ in diameter, comprised of mostly smooth, long branches with branching limited to the most proximal segments. They stratified narrowly within sublamina a or b of the inner plexiform layer and the sublaminal organization was correlated to their off- and on-centre response properties, respectively^{13,14}. Displaced ganglion cells are extremely rare in the rabbit retina and may not exist in the visual streak¹⁵, excluding the possibility that the orientation-selective cells were ganglion cells with unlabelled axons.

It has been proposed that orientation (and direction) selectivity of retinal neurons may be a reflection of their oriented dendritic fields^{16–18}. The dendritic symmetry of orientation-selective amacrine cells (Fig. 1e and f) is inconsistent with this notion. I have, however, found a second type of orientation-sensitive amacrine cell, termed orientation-biased, whose dendritic architecture may have an important functional role. Orientation-biased amacrine cells, like orientation-selective cells, preferred stimuli oriented either parallel or orthogonal to the visual streak, were either on- or off-centre, transient or sustained, and displayed DMPs of -43.0 ± 10.3 mV (Fig. 2). Biased amacrine cell responses were reduced as the stimulus was rotated, with the minimum amplitude response elicited by a moving or stationary slit of light orthogonal to the preferred orientation (maximum/minimum response ratio for all cells ($n=6$) = 4.2 ± 0.8) (Fig. 2b and e). Responses for biased cells, however, displayed no clear hyperpolarizations associated with the reduction in amplitude as the stimulus was rotated. Further, depolarization of the membrane up to $+70$ mV with current injection did not produce a 'masked' light-evoked hyperpolarization as would be the case for silent or shunting inhibition. Electrical modelling of current flow within other types of retinal

FIG. 2 Responses from two orientation-biased amacrine cells. Unless indicated, methods and figure conventions are the same as in Fig. 1. *a*, Response to a small spot of light, $350 \mu\text{m}$ in diameter, centred on the receptive field. On-centre response consists of a spike at light onset followed by a sustained plateau component. *b*, Response to a $95 \mu\text{m}$ -wide moving slit of light (1.3 mm s^{-1}). *c*, Flatmount-view drawing of amacrine cell from which responses in *a* and *b* were recorded. The asymmetric dendritic arbor is about $320 \mu\text{m}$ across the longest axis and stratifies within sublamina b of the IPL. *d*, Response of a different amacrine cell to a small spot of light, $350 \mu\text{m}$ in diameter. Off-centre response consisting of multiple spikes and slow potentials at light offset. *e*, Response to moving slit of light (1.3 mm s^{-1}) presented at four orientations. *f*, Flatmount drawing of HRP-labelled amacrine cell from which responses were obtained in *d* and *e*. A small dendritic arbor is emitted from the soma (outlined with dotted line) and branches in a nearby region of the IPL. However, a second, highly asymmetric dendritic arbor is also emitted from the bottom of the soma and runs for $350 \mu\text{m}$ and stratifies within sublamina a of the IPL. Amacrine cells with this unique dendritic morphology have been described previously in the rabbit retina²⁴. The somas of all orientation-biased amacrine cells ($n=6$) were found in the proximal region of the inner nuclear layer.



amacrine cells suggests that synaptic activity in distal dendrites can be isolated electrically from the soma^{19,20}. Thus, inhibition originating at distal synapses may not have been detected by microelectrodes placed in the soma. The receptive and dendritic fields of orientation-biased amacrine cells were, however, comparable in size, arguing that even distally located synaptic events were propagated effectively to the soma.

Morphologically, orientation-biased amacrine cells displayed more heterogeneity than did orientation-selective cells, in terms of soma size and dendritic architecture and they could not be placed into any single morphological class. However, a distinctive feature of all biased cells was the marked asymmetry of their dendritic arbors (Fig. 2c and f) and, moreover, the dendritic arbor of each cell was elongated along an axis which matched exactly its angle of preferred orientation (Fig. 2). The oriented dendritic fields may provide the structural basis for elongated receptive fields, which in turn forms the preference for oriented stimuli which can cover the maximum centre-receptive field area. Thus, consistent with the electrophysiological data, orientation-biased responses can be explained on anatomical grounds alone without the need for proposing inhibitory interactions.

The present findings refute the notion that complex response properties (for example, orientation and direction selectivity) in retina are unique to the ganglion cells and are formed by the interactions of bipolar and amacrine cells with 'simple' centre-surround receptive fields (for review, see ref. 21). An alternative hypothesis, supported by my data, is that ganglion cells receive orientation-sensitive information already created at the amacrine cell level. Indeed, orientation-selective amacrine and ganglion cells^{3,22} have a number of features in common. They are both found in the visual streak, are on- or off-centre, display broad orientation tuning and prefer stimuli oriented at either 0° or 90°. Cholinergic⁹ and GABAergic^{7,8} amacrine cell pathways

in the IPL subserve the formation of orientation selectivity of retinal ganglion cells. A question for future research is whether the morphologically homogeneous group of orientation-selective amacrine cells are incorporated into these pathways. Orientation-biased amacrine cells may provide a redundant mechanism, but their morphological heterogeneity suggests a more loosely organized system which may have a more subtle role in sending orientation-sensitive information proximally to ganglion cells. □

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Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter

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At most synapses, chemical signalling is terminated by a rapid reaccumulation of neurotransmitter into presynaptic terminals^{1–5}. Uptake systems for the biogenic amines are the initial site of action for therapeutic antidepressants and drugs such as cocaine and the amphetamines. We have isolated a complementary DNA clone encoding a human noradrenaline transporter. The cDNA sequence predicts a protein of 617 amino acids, with 12–13 highly hydrophobic regions compatible with membrane-spanning domains. Expression of the cDNA clone in transfected HeLa cells indicates that noradrenaline transport activity is sodium-dependent and sensitive to selective noradrenaline transport inhibitors. Transporter RNA is localized to the brainstem and the adrenal gland. The predicted protein sequence demonstrates significant amino-acid identity with the Na⁺/γ-aminobutyric acid transporter, thus identifying a new gene family for neurotransmitter transporter proteins. Analysis of its structure and function may lead to

structure-based drug design for the treatment of human depression and could help determine whether transporter abnormalities underlie affective disorders.

The noradrenaline transporter cDNA was isolated using an expression cloning strategy^{6–8}. Pools of clones from a human SK-N-SH cell⁹ cDNA library were transfected into COS-1 cells and transfectants expressing the noradrenaline transporter were identified. The noradrenaline analogue *m*-iodobenzylguanidine ([¹²⁵I]mIBG)^{10,11} is accumulated intracellularly by SK-N-SH cells through the noradrenaline transporter¹², allowing autoradiographic visualization of transporter-expressing transfectants. DNA was rescued from positive COS-1 transfectants by Hirt lysis¹³ and the resulting plasmid pools were rescreened and subdivided until a single clone was obtained.

Sequence analysis of the cloned cDNA indicates a 1,851-base pair (bp) open reading frame within a 1,983-bp insert (Fig. 1a). Assignment of the first ATG as the translation initiation site is based on its resemblance to the consensus sequence described by Kozak¹⁴. The sequence predicts a protein of 617 amino acids with a relative molecular mass of ~69,000 (*M*_r 69K). Hydrophobicity analysis¹⁵ of the noradrenaline transporter protein indicates that the molecule contains 12–13 hydrophobic segments each of 18–25 amino acids, which could form transmembrane domains. The absence of an identifiable signal sequence¹⁶ implies that the N terminus is located on the cytoplasmic face of the membrane. A tentative structural model (Fig. 1b) places the amino and carboxy termini in the cytoplasm and a large loop containing three potential glycosylation sites extracellularly, similar to the membrane topology proposed for the γ-aminobutyric acid (GABA) transporter¹⁷. In fact, the Kyte–Doolittle hydrophobicity plots of these two proteins are almost superimposable (data not shown).

Database comparisons demonstrate striking sequence similarity between the noradrenaline transporter and the rat¹⁷ and

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-60 GCGGGACACAGCCTCGGCGTGCCTCCAGGACCGTAAAGTTCCTCTCGCCAGCCGATCC -1
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21  P E Q P L R A R K T A E L L V V K E R N 40
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61  K K I D [F L L S V V G F A V D L A N V W 80
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1921 CAA 1923

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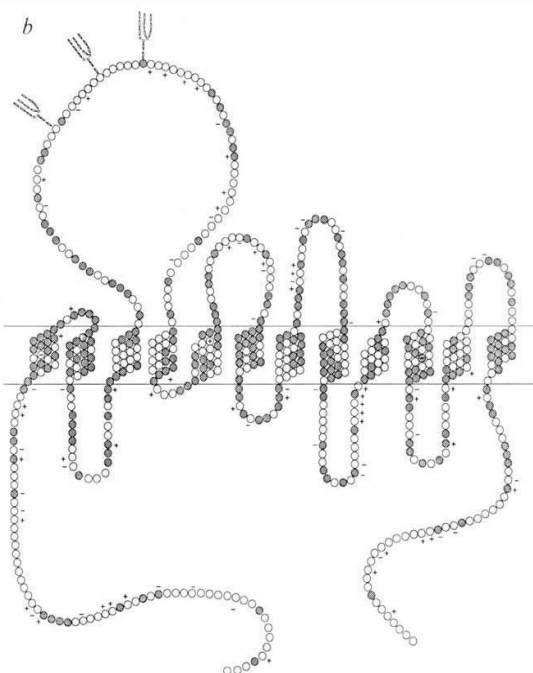


FIG. 1 a, Nucleotide and deduced amino-acid sequence of the human noradrenaline transporter cDNA. The first nucleotide and amino-acid residue of the translation start site are designated as position 1. Putative transmembrane domains predicted by the Kyte-Doolittle algorithm are bracketed and underlined. Motif resembling a leucine zipper, possibly a structure potentially mediating oligomerization of glucose transporters in the membrane³¹, is doubly underlined. Note the interspersed proline residues. Potential glycosylation sites on the putative extracellular loop are denoted by double underlining and a symbol. **b**, Schematic representation of the noradrenaline transporter showing proposed orientation in the plasma membrane. Darkened circles represent residues conserved with the GABA transporter. Glycosylation sites and charged residues are also denoted.

METHODS. COS cell functional screening: Poly(A) enriched mRNA was prepared from SK-N-SH neuroblastoma cells grown to confluence in 10-cm Petri dishes by the guanidinium isothiocyanate procedure³². Double-stranded cDNA was generated using random hexanucleotide primers for first-strand synthesis. Blunt-ended cDNA was ligated to semi-*Xho* adapters. Size fractionated (>1.5 kb) cDNA was ligated into the SV40-containing expression vector pXM. The ligation mixture was then electroporated into *Escherichia coli* DH10B bacteria to generate a library consisting of six pools ranging in size from ~20,000 to 50,000 independent clones, yielding a total library size of 250,000 colonies. Each pool was grown overnight and plasmid DNA was prepared by a double-banding caesium chloride procedure. Each plasmid pool was transfected (25 µg) into a 520 cm² square plate of 1.5 × 10⁶ COS-1 cells using DEAE-dextran (400 µg ml⁻¹) in DMEM + 10% Nu-serum. After 4 h, cells were shocked with 10% dimethyl sulphoxide in phosphate-buffered saline for 2.5 min³³. Cells were then incubated in DMEM + 5% fetal calf serum containing 100 µM chloroquine³⁴ for 4 h. Transfected cells were incubated for 48 h before performing the uptake assay. mIBG (20 nM), pre-purified over an AG 1-X8 anion-exchange resin column (200–400 mesh; chloride form) to remove free iodide, was added in DMEM + 5% fetal bovine serum for 90 min with gentle agitation at 37 °C. Cells were washed three times with DMEM and frozen at –70 °C. Plates were exposed to film for 48–72 h. Single-cell positives were picked by scraping an area of ~7 mm² of frozen cells from the plate and Hirt lysates were prepared as described¹³. DNA from these samples was then electroporated into *E. coli* DH10B bacteria and plated. Colony counts varied from 100 to 25,000; replica filters were stored at –70 °C. DNA from these pools was transfected into COS-1 cells and screened as before. Replica filters of positives were then sequentially subdivided and a single clone ultimately obtained. DNA sequencing³⁵; the noradrenaline transporter cDNA was excised from the *Xho*I site of pXM and cloned into Bluescript SKII(–) vector in both orientations and both strands were completely sequenced with Sequenase (US Biochem.) from a set of overlapping exonuclease III-digested *Sac*I, *Hind*III fragments. Two regions of poor resolution on one strand (from nucleotides 75 to 85 and from 1,685 to 1,690) were unambiguously resolved on the other strand.

human¹⁸ GABA transporters. No significant similarity exists with other proteins in the Genbank and NBRF Protein Identification Resource Files, including facilitated glucose carriers, Na⁺/glucose and Na⁺/proline carriers and adrenergic receptors. Overall identity between the amino-acid sequences of the human noradrenaline transporter and the human GABA transporter is 46%. Allowing for conservative amino-acid substitutions, the similarity between these two transporters increases to 68%. A region of particularly striking conservation occurs from amino acids 78 to 98, where 20 residues are identical, with a single nonconservative change at position 87. The homologies between the noradrenaline and the GABA transporters identify a new transporter gene family. They also suggest domains and residues critical to their common mechanisms, such as the coupling of the downhill movement of sodium and chloride ions³ to the accumulation of neurotransmitter against a concentration gradient. Regions of divergence may represent domains that are involved in more specific interactions with substrates and antagonists.

Noradrenergic neurons project diffusely to most parts of the mammalian brain from cell bodies in various brainstem nuclei, particularly the *locus coeruleus*. Tissue and cell-line expression of noradrenaline transporter messenger RNA was examined by northern blot analysis (Fig. 2). SK-N-SH cells and PC-12 cells¹⁹ contain two RNAs, of 5.8 kilobases (kb) and 3.6 kb, which hybridize to the noradrenaline transporter probe. The 5.8-kb species is selectively expressed in a brainstem region containing the *locus coeruleus* and in the adrenal gland. This parallels the distribution of noradrenergic cell bodies and thus it is likely that the larger mRNA encodes a neuronal noradrenaline transporter. The 5.8-kb mRNA is the predominant species in SK-N-SH cells and probably corresponds to the cloned cDNA. The nature of the ubiquitously expressed 3.6-kb RNA species is less certain. It could represent the transcript of a cross-hybridizing but distinct gene, or an alternatively processed transcript of the same gene, encoding an identical or related transporter. As desipramine-sensitive, Na⁺-dependent noradrenaline transport has been observed in primary cultures of neonatal rat astrocytes²⁰, the widely distributed 3.6-kb RNA might represent a glial-specific form of the transporter.

To determine the extent to which the cloned cDNA reproduces the uptake characteristics and pharmacology of endogenous carriers, an expression vector containing the noradrenaline

TABLE 1 Inhibitor sensitivity of l-noradrenaline uptake in HeLa cells transfected with noradrenaline transporter cDNA

Inhibitor	K_i (nM)
Mazindol	1.36
Desipramine	3.88
Nomifensine	7.68
Nortriptyline	16.5
D-Amphetamine	56.1
Imipramine	65.4
Amitriptyline	100
GBR 12909	133
Dopamine	139
Cocaine	140
Paroxetine	312
Benztrapine	822
Citalopram	>1000
Propranolol	10,000
Serotonin	>10,000

HeLa cells (200–300,000 per well) infected with a T7 RNA polymerase-containing vaccinia virus were transfected with the transporter in bluescript SKII (100 ng) and incubated with 20 nM [2, 5, 6, ³H]-l-noradrenaline (NEN) ± inhibitors for 15 min at 37 °C. The following substances had K_i values also in excess of 10 µM: reserpine, an inhibitor of vesicular uptake; nipecotic acid, an inhibitor of GABA transport; hemicholinium, an inhibitor of choline transport; phloridzin, an inhibitor of Na⁺-dependent glucose transport; prazosin, an α -1 adrenergic antagonist, and yohimbine, an α -2 adrenergic antagonist. K_i values reflect mean estimates from triplicate determinations of complete uptake inhibition curves, adjusting for substrate concentration after Cheng and Prusoff³⁰. Hill coefficients obtained for these data do not deviate significantly from unity.

transporter cDNA was transfected into HeLa cells and the uptake of [³H]-l-noradrenaline was monitored. Accumulation of the ligand demonstrates a marked sodium dependence; replacement of extracellular sodium by choline ions reduces noradrenaline uptake to levels of vector-transfected controls (Fig. 3a). Uptake is saturable, with a K_m of 457 nM (Fig. 3b). It is blocked by several well established inhibitors of noradrenaline uptake (Fig. 3c) with a rank order of potency identical to that observed in SK-N-SH cells and cortical synaptosomes^{12,21,22} (Table 1). Of the tricyclic antidepressants examined, desipramine and nortriptyline were the most potent antagonists of noradrenaline transport. Two highly specific inhibitors of serotonin transport, citalopram and paroxetine, are weak inhibitors of noradrenaline uptake. GBR 12909, a potent and specific inhibitor of the dopamine carrier²³ (K_i = 1 nM for ³H-dopamine uptake into striatal synaptosomes) is notably less potent at inhibiting noradrenaline uptake (K_i = 133 nM), and thus distinguishes the cloned activity from catecholamine transport activities characteristic of dopaminergic neurons.

Among the drugs with abuse potential, cocaine has a K_i of 140 nM. As this affinity is comparable to that observed in a variety of *in vitro* transport and binding studies, it seems that the noradrenaline transporter is the first cloned member of the 'cocaine receptor' family. The euphoric and addictive effects of cocaine seem to result from a blockade of the dopamine transporter in processes of ventral tegmental dopaminergic neurons²⁴. The noradrenaline transporter, however, mediates many systemic effects through an augmentation of sympathetic activity, resulting in acute changes in cardiac function and vascular tone.

Uptake of noradrenaline is also potentially inhibited by d-amphetamine (K_i = 56 nM)²⁵. Whereas cocaine is a non-transported inhibitor of the noradrenaline carrier, amphetamine is known to be a substrate for accumulation. Prolonged intracellular elevations of some transport substrates can eventually cause neuronal death. Some substrates for the noradrenaline transporter such as MPP⁺ (1-methyl-4-phenylpyridinium ion), DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine), and 6-OHDA (6-hydroxydopamine)^{26–28} are very potent neurotoxins. The anatomic specificity of their neurotoxicity

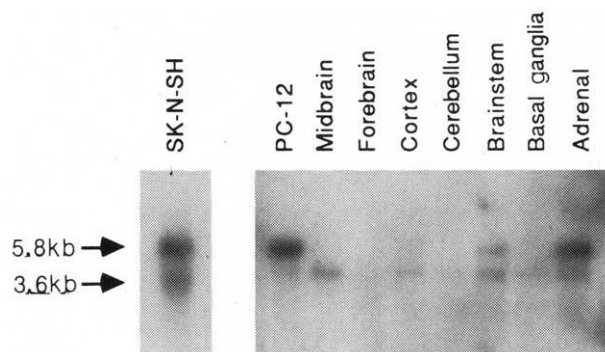


FIG. 2 Noradrenaline transporter RNA localization in human neuroblastoma cells (SK-N-SH), rat pheochromocytoma cells (PC-12), various rat brain regions and rat adrenal gland by northern blot analysis.

METHODS. Northern blots: poly(A)-enriched RNA was isolated from tissues (Fast-Track kit, Promega). Noradrenaline transporter cDNA (50 ng) excised from pXM was labelled by random primed synthesis using ³²P-labelled dCTP (100 µCi) using random oligonucleotides as primers. RNA (5 µg) was size-fractionated on a denaturing formaldehyde agarose gel and transferred to a nylon membrane (Zeta probe, BioRad) by vacuum blotting. The blot was hybridized for 48 h at 42 °C in 50% formamide, 5 × SSPE, 1 × Denhardt's solution, 10% dextran sulphate, 1% SDS, and 500 µg per ml salmon sperm DNA. The blot was washed at 65 °C for 1 h in 0.1 × SSPE buffer and 0.1% SDS. The SK-N-SH lane was exposed for 15 h and the other lanes for 48 h.

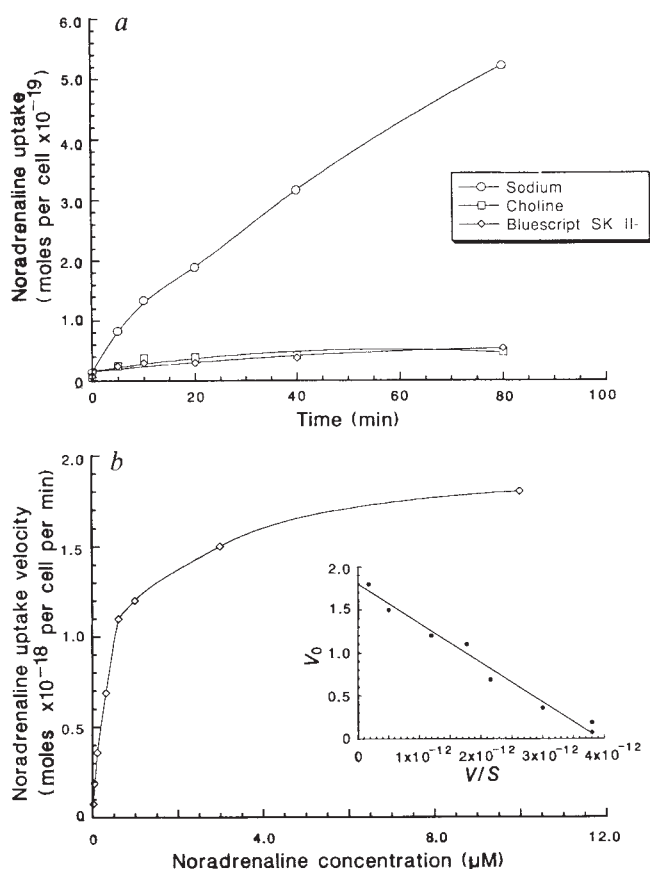
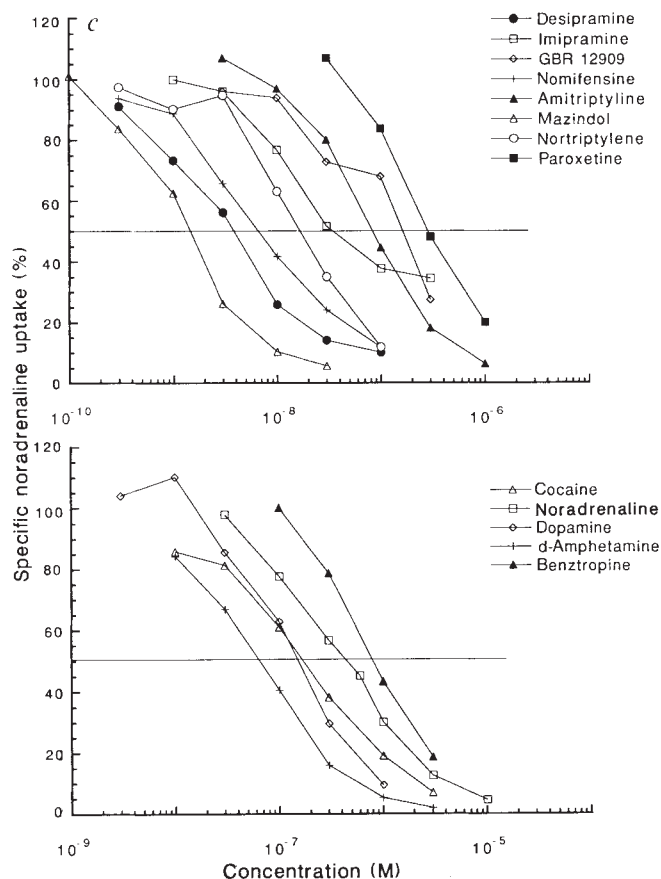


FIG. 3 *a*, Time course of [^3H]-noradrenaline accumulation into HeLa cells transfected with an expression vector containing the noradrenaline transporter cDNA, showing the effects of choline substitution for sodium, and background levels with vector only. *b*, Uptake velocity observed in the presence of various concentrations of substrate in transfected HeLa cells. Inset: Eadie-Hofstee analysis of initial velocity data. *c*, Inhibition curves depicting pharmacological sensitivity of noradrenaline uptake in transfected HeLa cells.

METHODS. HeLa cells cultured in DMEM + 5% FBS and penicillin/streptomycin (100 U ml^{-1}) were plated at $2-3 \times 10^5$ per well (24-well plates) and were



infected with T7 RNA polymerase-containing vaccinia virus³⁶ at a multiplicity of ~ 10 PFU per cell. After 30 min, cells were transfected with the noradrenaline transporter in bluescript SKII (100 ng) + bluescript (900 ng) using $3 \mu\text{g}$ lipofectin (BRL) reagent³⁷. After 12 h, cells were assayed for uptake. [2,5,6- ^3H]-noradrenaline ($43.7 \text{ Ci mmol}^{-1}$) at 10 nM (20 nM for competition curves) with $100 \mu\text{M}$ ascorbate and $50 \mu\text{M}$ pargyline was incubated with cells for 15 min in Krebs-Ringer-HEPES (KRH) buffer¹² containing either sodium chloride or choline chloride (120 mM) at 37°C . Uptake was terminated by three ice-cold washes with appropriate KRH buffer, cells were solubilized in 1% SDS and the radioactivity determined by scintillation counting.

in vivo is mediated by their relative affinities for different monoamine transporters. The availability of cDNAs encoding transporter proteins may facilitate the development of more sensitive screening techniques to identify environmental and other novel neurotoxins.

These studies demonstrate that a single cDNA effectively reconstitutes many properties of the native noradrenaline transporter, including an appropriate ion dependence and pharmacology. The antagonism of monoamine transport by antidepressants and subsequent elevation of synaptic neurotransmitter concentrations is an important component of the biogenic amine hypothesis of affective disorders. This proposes that a deficiency of particular amines at functionally important synapses results in depression²⁹. This model has shortcomings because, for example, reuptake blockade occurs shortly after administration, whereas clinical improvement from antidepressants may take weeks. Nonetheless, it is clear that noradrenaline and serotonin transporters are important initial targets for a number of tricyclic and other antidepressants and are involved in the complex cascade of synaptic and neuronal biochemical alterations that ultimately ameliorates depression. Determination of the structural basis for antidepressant binding may aid in the development of more selective therapeutic agents for the treatment of human depression. The human cDNA encoding the noradrenaline transporter also offers an opportunity to deter-

mine whether alterations in transporter genes could have aetiological implications for major psychiatric affective disorders.

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Glucose/galactose malabsorption caused by a defect in the Na⁺/glucose cotransporter

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GLUCOSE/galactose malabsorption (GGM) is an autosomal recessive disease manifesting within the first weeks of life and characterized by a selective failure to absorb dietary glucose and galactose from the intestine¹. The consequent severe diarrhoea and dehydration are usually fatal unless these sugars are eliminated from the diet. Intestinal biopsies of GGM patients have revealed a specific defect in Na⁺-dependent absorption of glucose in the brush border²⁻⁴. Normal glucose absorption is mediated by the Na⁺/glucose cotransporter in the brush border membrane of the intestinal epithelium⁵. Cellular influx is driven by the transmembrane Na⁺ electrochemical potential gradient; thereafter the sugar moves to the blood across the basolateral membrane via the facilitated glucose carrier. We have previously cloned and sequenced a Na⁺/glucose cotransporter from normal human ileum⁶ and shown that this gene, *SGLT1*, resides on the distal q arm of chromosome 22⁷. We have now amplified *SGLT1* complementary DNA and genomic DNA from members of a family affected with GGM by the polymerase chain reaction⁸. Sequence analysis of the amplified products has revealed a single missense mutation in *SGLT1* which cosegregates with the GGM phenotype and results in a complete loss of Na⁺-dependent glucose transport in *Xenopus* oocytes injected with this complementary RNA.

N.H. and R.H., sisters aged 5 and 3 yr, are the children of healthy Syrian parents who are second-degree relatives. Each child presented with severe diarrhoea and dehydration within the first days of life and was diagnosed with GGM. Maintenance thereafter on a glucose/galactose-free diet was successful. Oral tolerance tests revealed severe impairment in the intestinal absorption of D-glucose and D-galactose, while the absorption of D-xylose and D-fructose was normal. Glucose was not excreted in the urine and each patient had a normal duodenal biopsy.

Intestinal biopsy samples from N.H. and R.H. were used as a source of RNA for the production of cDNA by reverse transcriptase. Overlapping segments (Fig. 1a) of *SGLT1* messenger RNA were amplified by the polymerase chain reaction (PCR) from the cDNA of N.H. for sequence analysis. A single base change of a guanine (G) to an adenine (A) was discovered (Fig. 2a) at position 92. This corresponds to the first codon position of amino-acid residue 28 in the protein product, changing an aspartate to an asparagine. The G→A base change was also

found in the other afflicted sister, R.H. (Fig. 2a). The absence of a G signal at position 92 suggested that the two sisters were homozygous for the base substitution.

The entire coding region of cDNA from N.H. was sequenced to search for any other mutations. The N terminus, with its short 5' untranslated region, posed a special problem. The sequences of the promoter region and the first intron, obtained from a human genomic cosmid clone of the *SGLT1* gene (E.T. and E.M.W., in preparation), were used to design primers (segment E2, Fig. 1b) flanking the first exon for PCR-amplification from genomic DNA. Analysis of this PCR product completed the sequencing of the coding region and showed base 92 to be the only deviation from the published cDNA sequence of *SGLT1*⁶.

Genomic DNA of the parents was PCR-amplified (segment E1, Fig. 1b). The presence of both an A and G at position 92

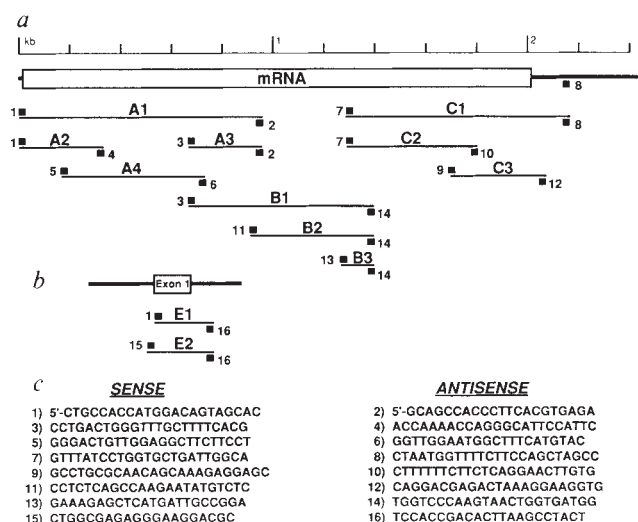
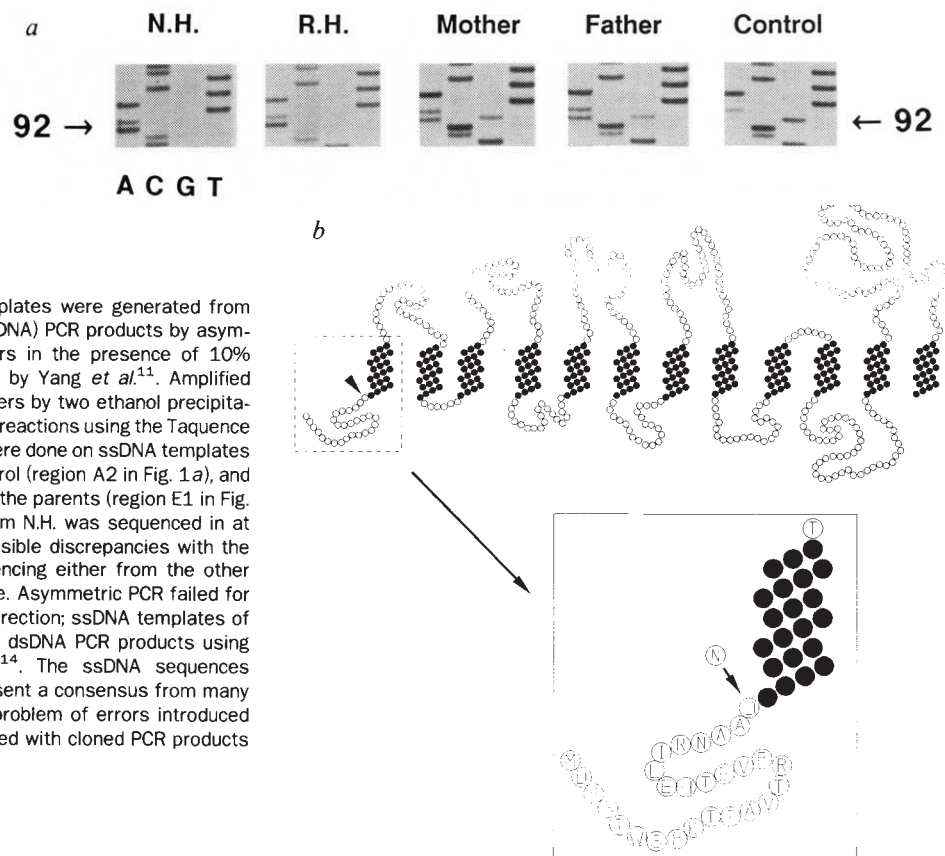


FIG. 1 PCR-amplification of the protein-coding region of *SGLT1* cDNA and genomic DNA from a family affected with GGM. a, Oligonucleotide primer pairs used to PCR-amplify overlapping segments of cDNA synthesized from RNA isolated from intestinal biopsy samples from N.H. and R.H. b, Primer pairs used to PCR-amplify most of exon 1 (E1) from genomic DNA of the parents, and all of exon 1 (E2) from genomic DNA of N.H. c, Primer sequences. METHODS. Duodenal 10 mg biopsies were taken 15 cm aboral the pylorus (small bowel at Teitz' ligament) from N.H. and R.H. using a Watson intestinal biopsy capsule. The parents gave their informed consent to all procedures and to the experimental studies. Pieces of the biopsies (4 mg) and a 20 mg biopsy of a normal ileum were used to prepare total RNA by a modified method¹⁰. Homogenization of tissue in 100 µl 4 M guanidinium thiocyanate, 1% β-mercaptoethanol, 10 mM EDTA, 100 mM Tris-Cl, pH 7.5, with 20 µg of carrier *Escherichia coli* ribosomal RNA was followed by several passes through a 23-gauge needle to shear DNA. After a 2-min centrifugation at 16,000g the supernatant was ultracentrifuged over a 75-µl cushion of diethylpyrocarbonate (DEPC)-treated 5.7 M CsCl, 10 mM EDTA at 100,000 r.p.m. for 2 h in a Beckman Airfuge. CsCl was extracted with 70% ethanol from the pellets which were then dried and dissolved in 20 µl DEPC-treated water. A 2-µl RNA aliquot was heated with 10 pmol oligonucleotide 8 (Fig. 1c) for 1 min at 70 °C, chilled in ice, and cDNA was synthesized in a 20 µl volume containing the denatured RNA and primer, 1 mM each of dATP, dCTP, dGTP and dTTP, 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-Cl, pH 8.3, at 25 °C, 10 mM dithiothreitol, 1 µg BSA, 19 U RNasin (Promega), and 200 U of Moloney murine leukaemia virus reverse transcriptase (BRL). Incubation was for 30 min at 42 °C followed by 15 min at 55 °C. Transcriptase was destroyed at 95 °C for 10 min. PCR-amplification of cDNA in an Ericomp (San Diego) TwinBlock comprised 30 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 57 °C (54 °C for segments C2 and C3), and elongation for 2-6 minutes at 76 °C. The reaction buffer was that described by Yang *et al.*¹¹ with 10% dimethyl sulphoxide, 50 µM each dNTP, 150 nM each of the two primers, 700 µM free (unchelated) Mg²⁺, 1 µl cDNA (or 1 µg genomic DNA) and 2 U *Taq* polymerase (Perkin-Elmer Cetus) in 100 µl. Genomic DNA was isolated from blood and was boiled for 3 min before amplification (42 cycles). Complete reaction mixtures under 60 µl oil were placed in the preheated TwinBlock at 95 °C for 60 s before beginning the first cycle. Gel purification of primers strikingly reduced primer-dimer interference.

FIG. 2 Identification of a missense mutation in two sisters, N.H. and R.H., with GGM. *a*, Sequence analysis at base 92 of GGM family members and an unaffected control. *b*, Localization of the Asp 28 → Asn 28 mutation on a twelve-span secondary structure model of the Na⁺/glucose cotransporter protein, predicted using the algorithms previously described^{6,12}. Transmembrane residues are shown filled in.

METHODS. Single-stranded DNA (ssDNA) templates were generated from agarose gel-purified double-stranded DNA (dsDNA) PCR products by asymmetric PCR¹³ with 1 μ M and 0.05 μ M primers in the presence of 10% dimethyl sulphoxide and the buffer described by Yang *et al.*¹¹. Amplified DNA was separated from unincorporated primers by two ethanol precipitations from 2 M ammonium acetate. Sequencing reactions using the Taq sequence kit (USB), primer 1 (Fig. 1c) and [α -³⁵S]-dATP were done on ssDNA templates from cDNA of N.H., R.H. and an unaffected control (region A2 in Fig. 1a), and on templates generated from genomic DNA of the parents (region E1 in Fig. 1b). The entire coding region of the cDNA from N.H. was sequenced in at least one direction. Ambiguous signals or possible discrepancies with the published sequence⁶ were resolved by sequencing either from the other direction or from a different segment template. Asymmetric PCR failed for regions C2 and C3 of Fig. 1a in at least one direction; ssDNA templates of C2 and C3 were generated alternatively from dsDNA PCR products using phosphorylated primers and λ exonuclease¹⁴. The ssDNA sequences obtained by each of these two methods represent a consensus from many original cDNA molecules, and thus avoid the problem of errors introduced by *Taq* polymerase amplification, as experienced with cloned PCR products (see legend to Fig. 4).



confirmed the parents' heterozygosity, and established that their children are homozygous for the mutation (Fig. 2a).

The base change detected in the two children destroys an *EcoRV* restriction site, allowing simple screening of other individuals. Southern blots of *EcoRV*-digested DNA from blood of 20 unaffected people and the four GGM family members were probed with a 5'-terminal fragment of the *SGLT1* cDNA. A 4.1-kilobase (kb) band was found in all 20 controls and the two parents, in whom the band was fainter, but not in the two sisters (Fig. 3). These results independently confirm that the children are homozygous for the mutation, whereas their parents are heterozygous. As this analysis was ambiguous for any possible heterozygotes among the 20 unaffected controls, exon 1 (segment E2, Fig. 1b) was PCR-amplified from them and the four GGM family members and analysed by *EcoRV* digestion. There were no heterozygotes among the 20 controls, whereas the two parents and children showed the restriction patterns expected of hetero- and homozygotes, respectively.

To determine whether the base change at position 92 is responsible for defective Na⁺/glucose cotransport, we examined the functional expression of the mutant *SGLT1* messenger RNA *in vivo*. The normal *SGLT1* clone, pG3, was converted by means of cassette mutagenesis to a new clone, pGM1, incorporating the G → A mutation at position 92. Capped mRNA was transcribed from both pG3 and pGM1 *in vitro* and injected into *Xenopus laevis* oocytes. The oocytes were later assayed for uptake of [¹⁴C]- α -methyl-D-glucopyranoside, which is a specific substrate for the Na⁺/glucose cotransporter³. The native form of the Na⁺/glucose cotransporter was found to stimulate α -methyl-D-glucopyranoside uptake 32-fold more than that of water-injected control oocytes (Fig. 4a). However, mRNA transcribed from the mutant clone pGM1 failed to elicit any detectable α -methyl-D-glucopyranoside transport activity above controls, indicating conclusively that the mutation Asp 28 → Asn renders the Na⁺/glucose cotransporter nonfunctional and is the direct cause of GGM in these patients.

In vitro translation experiments in the presence and absence of pancreatic microsomes (Fig. 4b) indicated that translation, membrane insertion and N-linked glycosylation were indistinguishable between the normal and mutant genes. Western analysis of brush border membranes isolated from three other patients showed the presence of the cotransporter, suggesting normal migration to the plasma membrane (B. A. Hirayama,

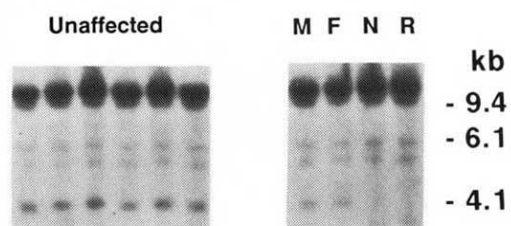
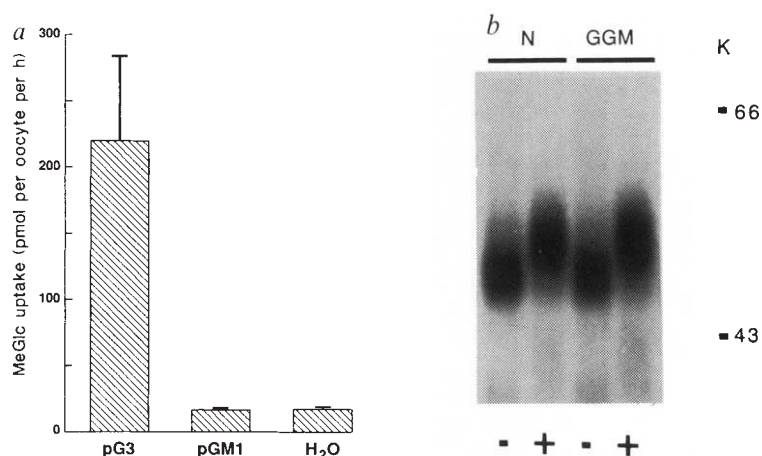


FIG. 3 Southern blot analysis of *EcoRV* restriction fragments of DNA from unaffected individuals and the GGM family members, probed with a 5'-terminal fragment of *SGLT1* cDNA. Six unaffected individuals are represented of the twenty analysed.

METHODS. Genomic DNA of unaffected controls was isolated from blood samples collected from E.M.W., E.T. and their colleagues at the University of California at Los Angeles. Genomic DNA (10 μ g) was digested with 48 U *EcoRV* at 37 °C for 1.2 h and electrophoresed on a 1% agarose gel at 50 V for 10 h. The gel was soaked in 0.5 M HCl, then in 0.5 M NaOH, 1.5 M NaCl, then neutralized in 0.5 M Tris-Cl, pH 7.5, 1.5 M NaCl and blotted onto nitrocellulose (BA 85, Schleicher and Schuell). DNA was crosslinked to the membrane with ultraviolet irradiation. The blot was prehybridized at 42 °C for 5 h in 50% formamide, 5 $\times$ SSC, 3 $\times$ Denhardt's solution, 25 mM NaMES, pH 6.5, 0.2% SDS, 10% dextran sulphate, 0.2 mg ml⁻¹ yeast RNA, and then probed overnight at 42 °C with the 5'-terminal *Bam*HI fragment of the pG3 insert (430 bp, labelled by Klenow fragment with [³²P]dCTP and random priming). The blot was washed with 5 $\times$ SSC, 0.1% SDS, 0.1% N-lauroyl sarcosine and 0.1 $\times$ SSC, 0.1% SDS at 65 °C before autoradiography.

FIG. 4 *a*, Uptake of α -methyl-D-glucopyranoside in *Xenopus* oocytes injected with cRNA from the native (pG3) and GGM (pGM1) forms of the Na⁺/glucose cotransporter. The normal *SGLT1* Bluescript clone, pG3, was converted by means of cassette mutagenesis, with a 280-bp *SacII*-*BsmI* fragment of a PCR-amplified cDNA product A1 (Fig. 1*a*) from N.H., to form a new clone which incorporated the G $\rightarrow$ A mutation at position 92. The new clone, pGM1, was sequenced through the PCR insert and found to contain the G $\rightarrow$ A conversion at position 92 and to be otherwise perfectly homologous to pG3. (An earlier, discarded construct had incorporated a 440-bp *SacII*-*BglIII* PCR insert which was found to have two errors, T $\rightarrow$ C base changes at positions 345 and 446, introduced by *Taq* polymerase amplification of cDNA, illustrating the need to verify the fidelity of cloned PCR products.) Capped mRNA was transcribed *in vitro* from *NotI*-linearized pG3 and pGM1 with T3 RNA polymerase (mCAP transcription kit from Stratagene) and 50 nl aliquots containing 40–50 ng mRNA or water were injected into oocytes from the same donor toad as described¹⁵. After incubation at 18 °C for 4 days, the oocytes were assayed for Na⁺-dependent uptake of 50 μ M [¹⁴C] α -methyl-D-glucopyranoside. Results are expressed in pmol per hour; error bars are s.e.m. The number of oocytes per group were 10 (pG3), 9 (pGM1), and 8 (H₂O). *b*, *In vitro* translation and glycosylation of the normal (N) and mutant (GGM) cotransporter mRNAs in the absence (–) and presence (+) of canine pancreatic microsomes. Capped



mRNA (400 ng) of pG3 and pGM1 was translated *in vitro*, in the presence of [³⁵S]methionine, in an mRNA-depleted rabbit reticulocyte lysate system (Promega) as described¹⁶, and analysed by fluorography after SDS-PAGE. N-linked glycosylation of the translation products¹⁷ was seen as an increase of 4,000 in the relative molecular mass.

S. Shirazi-Beechey, J. Walker-Smith and E.M.W., unpublished observations).

Secondary structure analysis⁶ of the *SGLT1* cotransporter places Asp 28 at the junction of the cytoplasmic N-terminal segment and the first transmembrane span (Fig. 2*b*). The function of this essential Asp 28 residue is unknown, but may include stabilization of tertiary structure by means of electrostatic interaction with a lysine or arginine on another cytoplasmic loop, participation in Na⁺ or sugar binding, or assisting in the conformational changes accompanying Na⁺/glucose cotransport.

Our findings are consistent with the autosomal recessive nature of GGM and indicate that a single missense mutation in *SGLT1* is responsible for GGM in this family. This is direct evidence that the Na⁺/glucose cotransporter encoded by *SGLT1* is the primary carrier species responsible for the intestinal uptake of glucose and galactose. To our knowledge, this is the first membrane transport protein in which a specific molecular defect has been found to be responsible for a human genetic disease. □

Potential of community-wide chemotherapy or immunotherapy to control the spread of HIV-1

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WHETHER zidovudine (3'-azido-3'-deoxythymidine, AZT) should be offered to symptomless individuals infected with human immunodeficiency virus type-1 (HIV-1), in the hope of delaying or even preventing progression to AIDS, has been much debated¹. The discussion has focused on the efficacy of the drug in delaying progression to disease², the severity of its side-effects³, and the likelihood of its prolonged and widespread use resulting in zidovudine-resistant strains of the virus⁴. Little attention has been given to the degree to which treatment reduces the infectiousness of symptomless patients, and to the concomitant implications for the overall transmission rate of HIV-1 in the community. Here we use simple mathematical models to show that community treatment with antiviral drugs or immunotherapies that lengthen the incubation period of AIDS without significantly reducing the infectiousness of treated individuals, can increase the rate at which HIV-1 infection spreads (which is fairly obvious) and can even, under certain circumstances, increase the AIDS-related death rate in the community (which is less obvious).

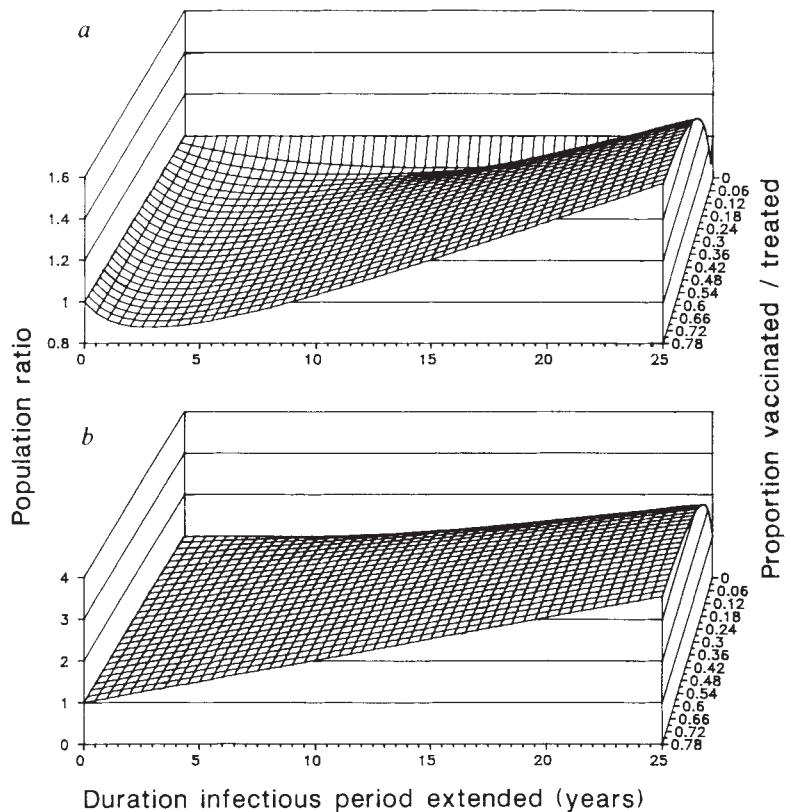
Zidovudine is a potent inhibitor of HIV replication, improving the survival chances of patients with advanced HIV-related disease (group IV of the Centers for Disease Control classification of HIV infection) or with AIDS², but inducing significant side-effects in some patients³. The drug is administered to symptomless patients in the hope that treatment will delay or even prevent progression to symptomatic disease and death. A randomized trial in the United States with asymptomatic patients and two dosage levels of zidovudine (500 mg or 1,500 mg per day) and a placebo was recently halted prematurely

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FIG. 1 The population ratio N^*/N_A , of equilibrium size (N^*) in the presence of treatment and/or vaccination (immunotherapy) divided by equilibrium size in the absence of control (N_A), plotted as a function of the degree to which treatment and/or vaccination increases the length of the infectious period ($1/(d+\mu) - 1/(v+\mu)$) and the proportion, g , of infecteds treated per unit of time ($g = 1 - \exp(-r)$). The calculations are designed to mimic the community-wide treatment with zidovudine of asymptomatics infected with HIV-1 ($s=0$, $p=0$, $r \neq 0$). For the model defined in the text, under these circumstances $N^* = [(1-\Psi)N_0]/[1-(1-\beta c)(\Psi/\phi)]$ where $\Psi = d\theta/(\mu+d) + (1-p)v/r + \mu + v$, $\phi = \theta/(\mu+d) + (1-p)/(r+\mu+v)$ and $\theta = p + (1-p)r/(r+\mu+v)$, with $N_A = (\mu N_0)/[(\mu+v)(1-v/\beta c)]$. In graphs *a* and *b*, $1/\mu = 30$ yr, $N_0 = 500,000.0$ and $1/(\mu+v) = 8$ yr (in the absence of treatment or immunotherapy the average incubation period of AIDS is ~ 8 yr). In *a*, $\beta c = 0.16 \text{ yr}^{-1}$ in *b*, $\beta c = 1.0 \text{ yr}^{-1}$. Treatment was not assumed to reduce infectiousness ($\beta_1 = \beta_2$). In *a*, $N^*/N_A < 1$ (the situation made worse by control) for low levels of community treatment (g small), particularly when the lengthening of the incubation period of AIDS induced by control is only a few years. In *b*, all levels of treatment improve the situation ($N^*/N_A > 1$).



(mean follow-up of 55 weeks) because patients showed a considerable reduction in rates of progression to AIDS or advanced AIDS-related complex (ARC), significant increases in the number of CD4 cells, and significant declines in levels of p24 antigen⁵. Zidovudine was concluded to be safe and effective in persons with asymptomatic HIV infection and fewer than 500 CD4⁺ cells per mm³. A similar trial in Britain and France (Concorde 1) is in progress and the results are eagerly awaited. In western Europe and North America, however, zidovudine is now widely used to treat patients with symptomless and symptomatic HIV infection.

But assuming treatment slows the rate of progression to AIDS and hence lengthens its incubation period, how does it affect the infectiousness of treated individuals? The measurement of infectiousness is difficult. Limited evidence suggests that infectiousness is positively correlated with levels of plasma viraemia and p24 antigenaemia⁶⁻⁸. High titres of virus and p24 antigens are more commonly observed in patients with symptoms of disease⁹ (in other words, during primary HIV infection or after the development of serious immunodeficiency), and horizontal or vertical transmission seems to be more likely when such symptoms are present¹⁰. Zidovudine treatment appears to improve p24 antigenaemia²⁻⁵ but only transiently—6 months to 1 year after the start of therapy the levels of free virus and antigen approach pre-treatment levels¹¹. The reasons for this are unclear, but could be due to the development of drug resistance, to transient dynamics after the perturbation induced by treatment¹² or to reservoirs of viral infection in immune-system cells. Whatever the cause, it seems that treated patients can soon become as infectious as untreated persons at the same stage of progression to AIDS. Thus even if treatment benefits the individual, it may not benefit the community if treated individuals remain infectious for longer than untreated individuals and continue to have unprotected sex with susceptible partners.

With simple mathematical models we have crudely assessed how wide-scale treatment of asymptomatics could affect the transmission of the virus. Previous models of HIV transmission

have been very detailed, encompassing distributed incubation and infectious periods, heterogeneity in sexual activity, networks of sexual contact, recruitment of susceptibles and changes in sexual behaviour^{13,14}. By contrast, we employ a simple model for a male homosexual population to facilitate analysis and make transparent our assumptions about community-based treatment. In our model, treatment includes the use of antiviral drugs and/or immunotherapy¹⁵ (which may prolong the effectiveness of drugs) that delay the onset of AIDS, but which cannot eliminate the virus from the patient.

The population is divided into susceptibles, X , infecteds who are neither vaccinated nor being treated with drugs, Y , vaccinated susceptibles, V_x , and infecteds who are vaccinated and/or being treated with an antiviral drug, V_y . We assume that vaccinated susceptibles (for example, those receiving immunotherapy) are not protected from infection. The model takes into account cohort immunization (that is, immunotherapy) of a proportion (p) of new recruits to the sexually active classes, immunotherapy applied to susceptibles at a constant per capita rate, and treatment of infecteds at a per capita rate r . The equations for the rate of change with respect to time of the four variables are:

$$dX/dt = \mu N_0(1-p) - (c\lambda + \mu + s)X;$$

$$dY/dt = c\lambda X - (v + \mu + r)Y;$$

$$dV_x/dt = \mu N_0 p - (c\lambda + \mu) V_x + sX;$$

$$dV_y/dt = c\lambda V_x + rY - (\mu + d) V_y,$$

where μN_0 is the net rate of recruitment of susceptibles to the sexually active age classes (N_0 is the population size of sexually active adults in the absence of infection; in this simplest model, we assume that recruitment is unaffected by the advent of HIV/AIDS); $1/\mu$ is the average duration of sexual activity of uninfecteds; c is the effective mean rate of change of sexual partners¹⁶; $1/(v + \mu)$ is the infectious period of untreated infecteds; and $1/(d + \mu)$ is the infectious period of treated and vaccinated infecteds. We assume that treatment or vaccination (that is, immunotherapy) prolongs the period from infection to

development of AIDS and death, so that $d < v$ and $1/(v + \mu) < 1/(d + \mu)$. We also pessimistically assume that sexual activity (c) is unaltered by treatment. The probability of acquiring infection from any one partner, λ , is given by $\lambda = (\beta_1 Y + \beta_2 V_y)/N$. Here β_1 and β_2 are the transmission probabilities (per partnership) from untreated and treated individuals, respectively. A pessimistic assumption is that treatment or vaccination has no effect on infectiousness ($\beta_1 = \beta_2$); more generally, we expect $\beta_1 \geq \beta_2$. N is the total population of sexually active adults. The classes Y and V_y contain infecteds and those with AIDS, so that the net death rate from AIDS in this community is $vY + dV_y$.

The model can embrace refinements such as differing rates of change of sexual partners¹⁷ for untreated (c_1) and treated (c_2) individuals and so can reflect the influence of counselling on treated persons ($c_1 > c_2$), of heterosexual transmission (two sexes represented by eight equations) and of heterogeneity in sexual activity (in the homosexual model, eight equations representing two sexual activity classes with low and high rates of partner change). But the essential conclusions follow from the four equations defined earlier. We use various quantities to measure the impact of treatment or immunotherapy: population size at equilibrium, N^* , relative to an untreated population, N_A , in which the virus is spreading; net death rate due to AIDS, $D = vY + dV_y$; and size of the infected population relative to an untreated community. All three are related, but the population-size ratio, N^*/N_A , is a simple measure: it will be >1 if community-wide treatment is beneficial, and <1 if detrimental.

Our conclusions are that control measures benefit the community (fewer deaths from AIDS each day) provided that

$$\beta c - (v + \mu) > v \quad (1a)$$

This result is for $\beta_1 = \beta_2$. More generally, if treatment reduces infectiousness ($\beta_1 > \beta_2$), then there is benefit to the community provided that

$$\beta_1 c - (v + \mu) > v \{1 - [(v + \mu)/(v - d)](\beta_1 - \beta_2)/\beta_1\} \quad (1b)$$

Failing this, control measures will eventually increase the rate of deaths from AIDS: infected individuals live longer, but there are more of them (owing to prolonged infectiousness), and the net effect is perverse. These expressions are approximations, which are valid either when $s = 0$, $p = 0$ and r small, or when $s = 0$, $r = 0$ and for any p ($1 > p > 0$).

The basic reproductive rate of infection, R_0 , defined as the average number of secondary cases of infection generated by one primary case in a susceptible population, is given by $R_0 = \beta c / (v + \mu)$. Hence equation (1) suggests that treatment or immunotherapy will benefit both the community and the individual, provided that R_0 is well in excess of unity. This point is made explicit in Fig. 1 where N^*/N_A is shown as a function of the degree to which drug treatment or immunotherapy increases the incubation period of AIDS ($[1/(d + \mu)] - [1/(v + \mu)]$) and the proportion (g) of infecteds treated per unit of time ($g = 1 - \exp(-r)$), for the pessimistic assumption that treatment does not decrease infectiousness ($\beta_1 = \beta_2$) or the rate

of change sexual partner ($c_1 = c_2 = c$). Two examples are plotted: curve a , $R_0 = 1.6$, and curve b , $R_0 = 8$. We note that for a , levels of treatment that increase the incubation period by only a few years make matters worse, whereas for b , all levels of treatment improve the situation, irrespective of the new incubation period. In both a and b , however, the numbers infected may be much greater with treatment than without, particularly if antiviral therapy or immunotherapy increases the incubation period by many years (because infected persons have a longer life expectancy) (Fig. 2).

Figures 1 and 2 are derived from a model with homogeneity in sexual activity. Figure 3 extends the analysis, exploring the effects of targeting drugs or immunotherapy to particular subgroups in a community having significant heterogeneity in rates of change of sexual partners¹⁷. As explained more fully in the legend, Fig. 3 shows the effect of treatment (measured by N^*/N_A) as a function of the degree to which it extends the incubation period, for various assumptions about how treatment is partitioned between groups of high and low sexual activity. If treatment or immunization is untargeted (that is, proportional to the representation of the class), the outcome is essentially similar to the simpler, homogeneous model. With targeting, however, the outcome is more complicated. For some sets of parameters in the range where R_0 is not too large (but >1), targeting treatment to the high-activity group can, for a fixed level of overall coverage, produce worse results than without targeting (see Fig. 3). This is because the high-activity group has a disproportionately large role in spreading infection to susceptibles in both low- and high-activity classes; lengthening the infectious period of individuals in this group therefore can result not merely in more infectious individuals, but also in more AIDS deaths each day, than when treatment is untargeted.

Our analyses suggest that antiviral therapy, although helping the individual (by delaying the onset of AIDS), can under certain circumstances harm the community (increasing the overall death rate from AIDS as a result of the additional infections produced by the lengthened infectious period). This perverse conflict between individual and group interests is not unique to the HIV-zidovudine issue. Certain levels of vaccination of young boys and girls against rubella virus can increase the incidence of congenital rubella syndrome in infants born to mothers who become infected during pregnancy—viral transmission is reduced, so more women of childbearing age become infected than before the introduction of immunization¹⁸. But drug treatment and/or immunotherapy for HIV is a more complex and less well understood issue. First, the populations at risk are greatly heterogeneous in behaviour, increasing the likelihood of infection. Second, the ability of zidovudine to suppress infectiousness is unknown. Third, and most important, it is unethical to refuse treatment to individuals, given that AIDS is lethal and that zidovudine might slow progression to AIDS.

Analyses that highlight the possible disadvantages of community treatment serve two purposes. First, they draw attention to the need to link counselling (such as 'safe sex') to treatment,

FIG. 2 A plot of the proportion infected ($y = (Y + V_y)/N$) as a function of proportion treated with zidovudine ($s = p = 0$, $r \neq 0$), g , and the degree to which treatment extends the incubation (infectious) period of AIDS. Parameter values as for Fig. 1, except $\beta c = 0.2 \text{ yr}^{-1}$. Note that treatment increases the proportion infected, y , in the total population, N .

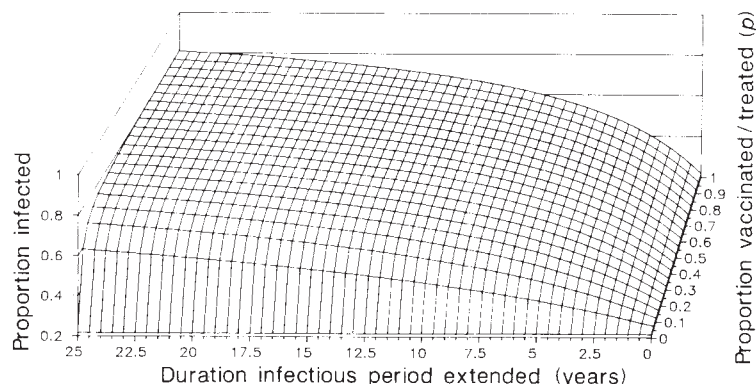
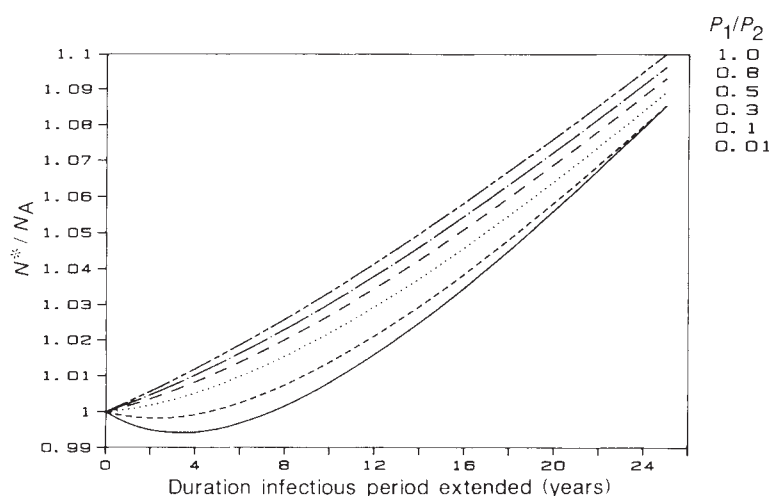


FIG. 3 The influence of targeting treatment to classes of high sexual activity. The graph records the population ratio N^*/N_A as a function of the degree to which treatment and/or vaccination extends the infectious (incubation) period of AIDS in infected persons ($1/(d+\mu) - 1/(v+\mu)$) and the degree to which treatment is targeted to classes of low (Y_1) or high (Y_2) sexual activity. The proportion, p_i , treated ($s=r=0, p \neq 0$) in the total population was set at 0.1 and the fraction receiving immunotherapy in each class (p_1 in the low-activity class and p_2 in the high-activity class) was varied. The different lines record the ratio p_1/p_2 with the top line denoting $p_1=p_2$ (no targeting) and the bottom line $p_1=0.01p_2$ (highly targeted) with intermediate levels in between. Note that a high degree of targeting makes matters worse (that is, ratio N^*/N_A becomes smaller) than without targeting. The model used to generate the calculations divided each susceptible and infected class on the basis of sexual activity, i (defined by the mean rate of acquisition of new sexual partners, c_i , in class i) where $dX_i/dt = (1-p_i)\gamma_i\mu N_0 - (c_i\lambda + \mu)X_i$; $dY_i/dt = c_i\lambda X_i - (\mu + v + r)Y_i$; $dV_{xi}/dt = p_i\gamma_i\mu N_0 - (c_i\lambda + \mu)V_{xi}$; and $dV_{yi}/dt = c_i\lambda V_{xi} + rY_i - (\mu + d)V_{yi}$. Here γ_i denotes the fraction of new recruits to the sexually active population that belong to class i . The term p_i is the fraction of class i receiving immunotherapy, which lengthens the incubation (infectious) period of AIDS (the calculations are here set to mimic immunotherapy as opposed to treatment with zidovudine). For this model, $N^* = N_0[1 - \lambda^* \Sigma [c_i\gamma_i\Psi_i / (c_i\lambda^* + \mu)]]$. For a two sexual-activity-class model ($i=1 \dots 2$), $\lambda^* =$



$\{-[qb] \pm [(qb)^2 - 4(qa)(qc)]^{1/2}\} / 2qa$, where $qa = \sigma_1\Psi_1c_2 + \sigma_2\Psi_2c_1 - cc_1c_2$, $qb = \mu\beta[\sigma_1\phi_1c_2 + \sigma_2\phi_2c_1] + \mu[\sigma_1\Psi_1 + \sigma_2\Psi_2] - \mu c[c_1 + c_2]$, $qc = \mu_2\beta \times (\sigma_1\phi_1 + \sigma_2\phi_2)$, given $c = \Sigma \gamma_i c_i$, $\sigma_i = c_i^2 \gamma_i$, $\phi_i = p_i/(\mu + d) + (1-p_i)/(\mu + v)$, $\Psi_i = d p_i/(\mu + d) + v(1-p_i)/(\mu + v)$. Parameter values: $p=0.1$; $\beta c=0.3 \text{ yr}^{-1}$; $\beta_1=\beta_2$; $\gamma_1=0.8$; $\gamma_2=0.2$; $c_1=1.26 \text{ yr}^{-1}$; $c_2=9.96 \text{ yr}^{-1}$; $1/(\mu + v)=8 \text{ yr}$; $1/\mu=30 \text{ yr}$; $N_0=500,000.0$.

with the aim of changing behaviours that facilitate transmission to susceptible sexual partners. Calculations based on the assumption that treated persons reduce their sexual activity ($c_1 > c_2$) show that the degree to which activity must be reduced to negate the detrimental impact of community-wide drug application depends critically on the magnitudes of the other parameters. For R_0 values just >1 , a 50% reduction in c can eliminate the detrimental effects on the community. Second, the analyses emphasize the need in clinical trials (which test efficacy and toxicity) to assess the degree to which antiviral drug treatment or immunotherapy suppresses infectiousness.

Although our models are simple, and should be refined as more information becomes available, the main conclusions seem to be robust. In communities where the transmission rate of HIV is low, but sufficient for long-term persistence (R_0 not much larger than unity), treatment that lengthens the infectious period is likely to be able to increase overall transmission rates to more than counterbalance the greater longevity of infected individuals who are treated. The result is a perverse increase in the death rate from AIDS. This situation could apply to some heterosexual communities. When transmission rates are high (as in most communities using intravenous drugs and in some male homosexual populations), our models suggest that community-wide treatment is always beneficial for both individuals and the community (although not necessarily for linked communities, such as heterosexual networks coupled by bisexuals to male homosexual groups). By emphasizing the possible effects of zidovudine at the population-level, we hope to have drawn further attention to the need to improve counselling aimed at reducing high-risk behaviours. □

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A Tyr/Ser protein phosphatase encoded by vaccinia virus

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PROTEIN tyrosine phosphorylation is associated with alterations in receptor activity, cellular proliferation and modulation of the cell cycle^{1,2}. Inappropriate tyrosine phosphorylation can lead to unrestrained cell growth and oncogenesis. Enzymes important in tyrosine dephosphorylation have also been described^{3,4-6}. Protein tyrosine phosphatases (PTPases) consist of two families. There is a receptor-like family of PTPases with an extracellular domain, transmembrane-spanning region and typically two repeated phosphatase domains⁷⁻¹¹. Proteins of the non-receptor-like family have a single catalytic phosphatase domain^{3,12-15}, show a substrate specificity for Tyr phosphate and will not hydrolyse Ser or Thr phosphate. Here we report that the vaccinia virus genome contains an open reading frame which shares amino-acid sequence identity with the PTPases^{3,12-16}. The purified protein encoded by the vaccinia virus *H1* open reading frame expressed in bacteria hydrolyses substrates containing phosphotyrosine and phosphoserine. Mutagenesis of an essential Cys in the vaccinia phosphatase abolishes catalytic activity directed towards both substrates, suggesting that hydrolysis proceeds by a common mechanism. Understanding the function of the *H1*-encoded protein will help to define the role of the phosphatase in viral replication and pathogenesis.

Recently, we demonstrated the existence of a PTPase in *Yersinia*¹⁷, the causative bacterial agent in the plague or the black death. The *Yersinia* PTPase gene is essential for bacterial pathogenesis¹⁸⁻²⁰. We thought that other pathogenic organisms might also exploit the tyrosine phosphorylation-dephosphorylation pathway. Using the highly conserved amino-acid sequence

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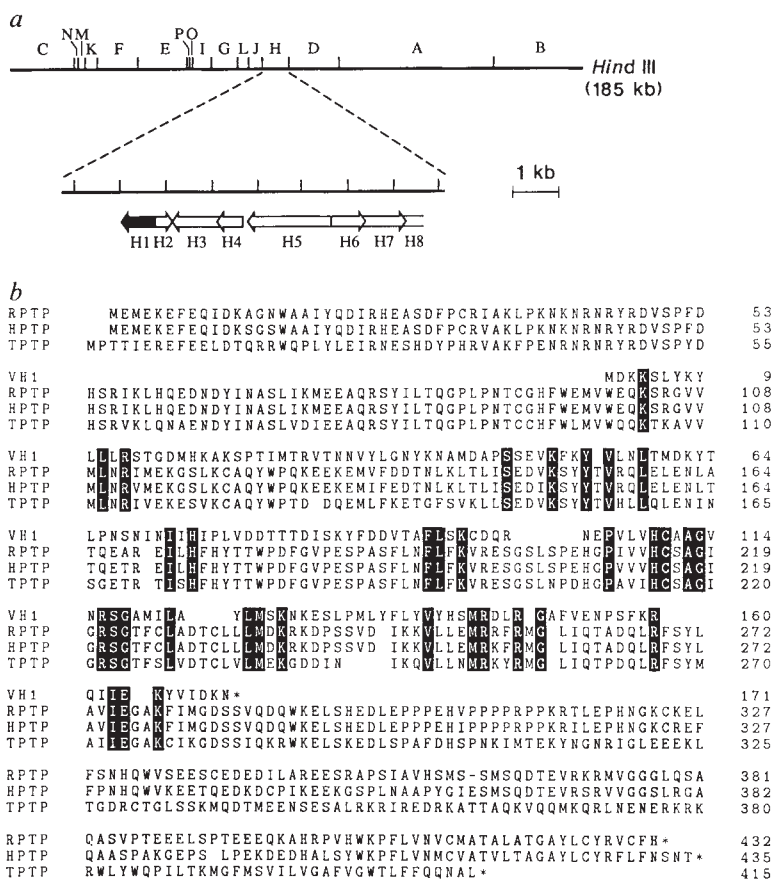


FIG. 1 *a*, Genomic map of vaccinia virus. The *Hind*III restriction map of vaccinia virus was adopted from Moss^{16,30}. The open reading frames of the *Hind*III H fragment are shown in detail. Filled arrow, open reading frame for VH1. *b*, Sequence alignment of VH1^{16,22} with rat brain PTP-1 (RPTP)¹⁵, human placenta PTP-1B (HPTP)^{12,13} and T-cell PTP (TPTP)¹⁴. The residues that are completely conserved are indicated by a black background. METHODS. Sequences were aligned using a combination of the FASTA and BESTFIT²¹ programmes. For maximum alignment, gaps were introduced. *, end of sequence.

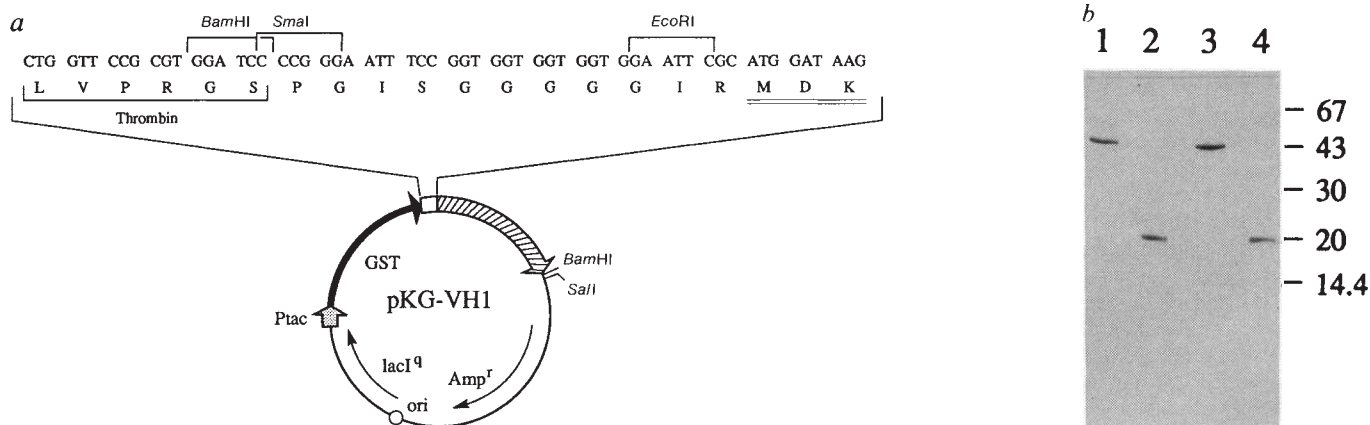


FIG. 2 Expression of recombinant vaccinia phosphatase. *a*, The plasmid pKG-VH1 was used to express VH1. Filled arrow, coding sequence for GST. Hatched arrow, coding sequence for VH1. Ptac, *tac* promoter. Open bar, thrombin cleavage site (cleavage occurs between the arginine and glycine residue) and the 'glycine kinker', with sequence information shown above the plasmid. The amino-acid sequence is shown underneath the nucleotide sequence. The double underlined amino acid residues indicate the N terminus of VH1. *b*, SDS-PAGE of purified proteins. *M*_s of standards (K) are shown on the right side of the gel. Lane 1, GST-VH1; lane 2, VH1; lane 3, GST-VH1 (C110S); lane 4, VH1 (C110S).

METHODS. Plasmid PH1 (refs 16, 22), which contains the open reading frame of VH1, was used as a template in polymerase chain reaction (PCR). Two primers were used in PCR of the *Vh1* gene. The 5' primer was (5'ATCCGA-ATTCGATGGATAAGAAAGTTTG) and the 3' primer was (5'CGTCACGGATCC-AGACTTTTAATCTTATC). PCR was performed at 94 °C for 1 min., 45 °C for 2 min., and 72 °C for 2 min for 2 cycles and at 94 °C for 1 min, 50 °C for 2 min and 72 °C for 2 min for 30 additional cycles. The PCR product was

digested with *Eco*RI and *Bam*HI and electrophoresed on an agarose gel. A fragment of ~0.5 kb was recovered and inserted into the *Eco*RI/*Bam*HI-digested vector pT7-7 to produce pT7-VH1. A 0.5-kb *Eco*RI-*Sal*I fragment (*Sal*I is located within the polylinker of pT7-7 adjacent to the 3' end of VH1) was isolated from pT7-VH1 and inserted into *Eco*RI/*Sal*I-digested pGEX-KG to produce pKG-VH1. Plasmid pKG-VH1 was used to express VH1 as a fusion protein with GST. Expression and purification of VH1 was performed as described²³. Purified proteins were analysed by SDS-PAGE. Approximately 5 mg per litre of bacterial culture of GST-VH1 were routinely obtained. Site-directed mutagenesis was done by taking the *Eco*RI-*Bam*HI fragment from pKG-VH1 and subcloning it into M13mp18. Codon TGT for Cys 110 was mutated to TCT for Ser using oligonucleotide-directed mutagenesis. The mutation was confirmed by DNA sequencing. The mutated DNA was subcloned into *Eco*RI/*Xba*I-digested pGEX-KG. Expression and purification of VH1 (C110S) mutant was carried out as previously described²³. After purification of VH1 and VH1 (C110S), glycerol was added to ~40%. The enzyme was stored at -20 °C.

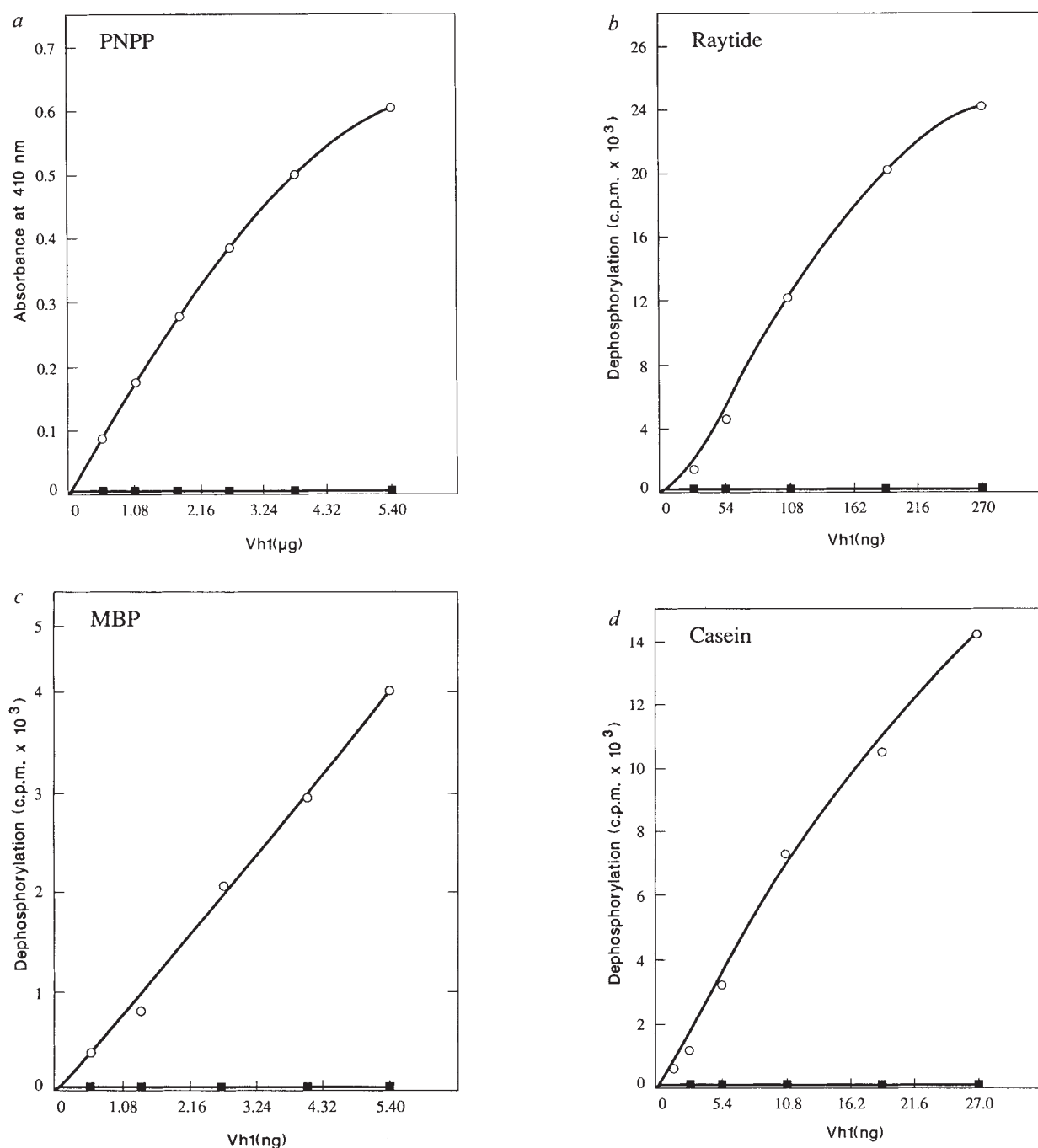


FIG. 3 Phosphatase activity of purified VH1 and VH1 (C110S). $\circ$, VH1; $\blacksquare$, VH1 (C110S). **a**, Hydrolysis of *p*-nitrophenyl phosphate (PNPP). **b**, Dephosphorylation of Raytide. **c**, Dephosphorylation of myelin basic protein (MBP). **d**, Dephosphorylation of casein.

METHODS. Hydrolysis of PNPP was done in 200 μ l of solution containing 50 mM imidazole, pH 7.5, 0.1% β -mercaptoethanol, 10 mM PNPP and enzyme at room temperature for 10 min. The reaction was stopped by addition of 800 μ l 0.25 M NaOH. Absorbance at 410 nm was measured. Raytide was phosphorylated on tyrosine by p43^{v-abl} (Oncogene Science) as follows: 10 μ g Raytide (Oncogene Science), 50 mM HEPES buffer, pH 7.5, 10 mM MgCl₂, 0.067% β -mercaptoethanol, 0.05 mM ATP including 300 μ Ci [γ -³²P]ATP, 4 U p43^{v-abl} kinase in a final volume of 60 μ l. The reaction proceeded at 30 °C for 30 min and was stopped by addition of 240 μ l 10% phosphoric acid. The sample was spotted onto two 1 $\times$ 1 cm P81 sheets of phosphocellulose paper and extensively washed with 0.5% phosphoric acid. Phosphorylated Raytide was eluted twice with 1 ml 500 mM (NH₄)₂CO₃, lyophilized and resuspended in 100 μ l H₂O. Phosphorylation on bovine MBP (Sigma) was similar to that for Raytide, except that MBP (30 μ g) was used instead of 10 μ g Raytide in each phosphorylation reaction. Ice-cold trichloroacetic acid (TCA) (40 μ l of a 50% solution) was used to precipitate MBP on ice for

30 min. After centrifugation, MBP was washed three times with 20% TCA and dissolved in 100 μ l H₂O. Phosphorylation of casein was done using the catalytic subunit of protein kinase A. Casein, 200 μ g, was phosphorylated in 200 μ l solution containing 40 mM Tris, pH 7.5, 20 mM magnesium acetate, 0.2 mM ATP including 300 μ Ci [γ -³²P]ATP, and 10 μ g catalytic subunit protein kinase A at 30 °C for 30 min. Phosphorylated casein was precipitated by addition of TCA to 20%, washed with 20% TCA to remove unincorporated [γ -³²P]ATP, and dissolved in 100 μ l H₂O. Dephosphorylation was performed in 20 μ l 50 mM imidazole, pH 7.5, 0.1% β -mercaptoethanol, purified enzyme and phosphorylated substrate, at room temperature for 10 min. Because of the low stoichiometry of phosphorylation on substrates, concentration referred to the actual phosphorylated substrate concentration. Phospho-Raytide or MBP (each at 10 nM) or 100 nM phospho-casein was used. Quantitation of dephosphorylation of Raytide was determined by a published method⁹. When MBP or casein was used as substrate, 20 μ l of the dephosphorylation reaction were added to 10 μ l BSA (10 mg ml⁻¹) and 180 μ l 20% ice-cold TCA to stop the reaction and precipitate protein. ³²P in the soluble fraction was counted to measure the amount of dephosphorylation. Reactions without VH1 were used as the negative control.

surrounding the active site of receptor and non-receptor-like PTPases (GPIVVHCSAGVGRGTG in single-letter amino-acid code), we searched the National Biomedical Research Foundation data bank for other proteins having the conserved PTPase active-site sequence²¹. Numerous members of the PTPase family, the *Yersinia* PTPase and a protein encoded by an open reading frame in vaccinia virus were identified^{16,22}. The open reading frame in the vaccinia virus genome, designated *H1*, is shown in Fig. 1a. The protein expressed from the *H1* gene appears in the late stage of viral infection²² and its function is unknown.

Sequence alignment of the vaccinia *H1* gene product (VH1) with the non-receptor-like PTPases^{3,12-15} is shown in Fig. 1b. There is ~20% amino-acid identity with the PTPases. The VH1 protein is small (relative molecular mass 20,000 (M_r , 20K)) compared with other non-receptor-like PTPases which have an M_r of ~50K. This may reflect efficient use of the compact virus genome. The stop codon in the *Vh1* gene is near the stop codon in the *Yersinia*¹⁷ and yeast PTPase (K.G., R. J. Deschenes, H. Qiu and J.E.D., unpublished results). It has been suggested that the mammalian PTPases have two domains, an N-terminal catalytic domain and a C-terminal domain important in regulation and/or targeting of the protein to the membrane¹⁵.

To determine whether VH1 possesses tyrosine phosphatase activity, the *Vh1* gene was isolated using the polymerase chain reaction. The 0.5-kilobase coding fragment of *Vh1* was subcloned into the vector pGEX-KG and expressed as a fusion protein with glutathione-S-transferase (GST) (Fig. 2a). The fusion protein, GST-VH2, was rapidly purified under non-denaturing conditions on a glutathione agarose affinity column²³. VH1 was cleaved from the fusion protein by thrombin. Both GST-VH1 and VH1 were obtained in a highly purified form as is evident from SDS-PAGE (Fig. 2b). The purified VH1 and GST-VH1 proteins were examined for their ability to dephosphorylate *p*-nitrophenyl phosphate, the phosphotyrosine containing peptide, Raytide, and myelin basic protein. The latter two were specifically phosphorylated on tyrosine using $p43^{v-abl}$ kinase. VH1 hydrolyses *p*-nitrophenyl phosphate rapidly (Fig. 3a). Purified GST-VH1 is also active and has a specific activity comparable to VH1 (data not shown). The rates of reaction are dependent on the time of incubation and substrate concentration (data not shown). The hydrolysis of phosphate from phosphorylated myelin basic protein and Raytide are shown in Fig. 3b and c. Both substrates can be completely dephosphorylated by either VH1 or the corresponding fusion protein. We also examined casein, phosphorylated by the catalytic subunit of protein kinase A, as a substrate. VH1 was not expected to dephosphorylate Ser- or Thr-containing substrates as other PTPases show a specificity for tyrosine phosphate^{4,15,17}. Dephosphorylation of Ser-phosphorylated casein is shown in Fig. 3d. To verify that the phosphoamino acid residue in casein was not Tyr, phosphoamino acid analysis was performed. Ser was the only residue phosphorylated in casein whereas phosphotyrosine was recovered from Raytide and myelin basic protein (data not shown). At 0.5 μ M substrate, Ser-phosphorylated casein was dephosphorylated by VH1 seven times more rapidly than Tyr-phosphorylated Raytide. Kinetic studies are underway to define the kinetic parameters of Ser- and Tyr-dephosphorylation.

Cys 215 in the rat brain PTPase and the corresponding Cys residues in the receptor-like PTPases and the *Yersinia* phosphatase are essential for catalytic activity^{9,17,24}. We used site-directed mutagenesis to alter Cys 110 in VH1 to Ser. The mutant VH1 (C110S) was expressed in the pGEX-KG vector and the fusion protein was obtained in a highly purified form (Fig. 2b). This protein was cleaved by thrombin, and the activities of the fusion protein and cleavage product were examined. Neither GST-VH1 (C110S) nor VH1 (C110S) displayed detectable activity towards phosphorylated Raytide, myelin basic protein, or casein (Fig. 3). This demonstrates the importance of Cys 110 in the hydrolysis of both Tyr and Ser phosphoproteins. The

hydrolysis of both substrates probably proceeds by a common catalytic mechanism using an essential Cys residue.

PTPases show a specificity for tyrosine phosphate hydrolysis and have a 'signature sequence' at their active site, namely HCXAGXXR. The mechanism of this family of phosphatases proceeds through a phospho-thiol intermediate involving the Cys in the signature sequence (K.G. and J.E.D., unpublished results). Type-2 Ser/Thr phosphatases also have residual tyrosine phosphatase activity²⁵⁻²⁸ which is dependent upon divalent cations. To verify that VH1 does not require a divalent cation for catalysis, VH1 activity was examined in the presence of EDTA. The Tyr or Ser phosphatase activity of VH1 is not inhibited by 10 mM EDTA. Okadaic acid, a Ser/Thr phosphatase inhibitor with 50% inhibitory concentration (IC_{50}) of 20 nM and 0.2 nM for type 1 and 2A enzymes respectively, was also tested²⁵. No inhibition of either Tyr or Ser phosphatase activity of VH1 was observed at concentrations of up to 1 μ M okadaic acid. In contrast, 1 mM sodium vanadate completely inhibits the activity of VH1.

Cohen and Cohen discovered a Ser/Thr protein phosphatase encoded by bacteriophage λ ²⁹, and suggested that the λ protein phosphatase may regulate production of viral RNA and protein. Although the function of the vaccinia phosphatase is unknown, it could have a pronounced effect upon tyrosine and/or serine phosphate content in cells infected with the virus. The vaccinia phosphatase may also play an important part in viral replication. In either case, it would seem that this virus, as well as pathogenic bacteria (*Yersinia*), probably exploit the phosphorylation-dephosphorylation signal transduction pathway by altering the extent of cellular protein phosphorylation. This may contribute to the pathogenic effects of these infections. The potential importance of the phosphatases in diseases caused by the family of pox viruses and the bubonic plague underscore the importance of further understanding events associated with the molecular pathogenesis of the PTPases. □

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